Environment Canada Environnement Canada

TRACER EXPERT WORKING GROUP FINAL REPORT

RECOMMENDATIONS FROM CYCLE 1 REVIEW

PREPARED FOR THE
EEM TECHNICAL MANAGEMENT COMMITTEE

EEM/1997/2

Canada

Publications relating to Environmental Effects Monitoring (EEM):

Aquatic Environmental Effects Monitoring Requirements (revised EPS 1/RM/18). Environment Canada. 1997. EEM/1997/1

Pulp and Paper Aquatic Environmental Effects Monitoring Requirements. Environment Canada. 1997. Annex 1 to EEM/1997/1

Tracer Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/2 is also published as: Ali, N et al, 1997. Exposed or not exposed: the effectiveness of using tracers to verify exposure of fish to pulp and paper mill effluent. Canadian Technical Report of Fisheries and Aquatic Sciences 2162.

Dioxin Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/3

Usability Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/4

Toxicology Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/5

Fish Survey Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/6

Benthic Community Expert Working Group Report. EEM Technical Management Committee. 1997. EEM/1997/7

Pulp and Paper Technical Guidance for Aquatic Environmental Effects Monitoring. 1997. EEM/1997/8

Copies are available at:

National EEM Office
Science Policy and Environmental Quality Branch
Environmental Conservation Service
Environment Canada
Ottawa, Ontario, Canada
K1A 0H3
Tel.: (819) 997-1535
Fax: (819) 953-0461
Email: eem@ec.gc.ca
Website: http://www.ec.gc.ca/eem/english/default.htm

103-05635/

Note

The contents of this report are the views of the members of the Environmental Effects Monitoring (EEM) Expert Working Group. As well, the recommendations in this report are the result of the Expert Working Group process and represent the conclusions of the Expert Working Group.

The recommendations and decision trees put forward in this report will be submitted to the EEM Technical Management Committee (TMC). This information will be used by the TMC to produce the TMC recommendations for updates to the regulatory requirements of the EEM program.

The documents which will contain the revised regulatory requirements will be called:

- Aquatic Environmental Effects Monitoring Requirements, EEM/1997/1
- Pulp and Paper Aquatic Environmental Effects Monitoring Requirements, ANNEX 1 to EEM/1997/1

Acknowledgments

The EEM Technical Management Committee (TMC) extends its thanks to the members of the Expert Working Groups for their contribution to the EEM program. The members of the group have produced their reports in a very tight time frame and they have displayed a willingness to work together to meet common objectives.

The review performed by the Expert Working Groups and their recommendations for enhancements to the program are essential ensuring that the EEM program remains based on sound science.

Table of Contents

	ABSTRACT	iv
	EXECUTIVE SUMMARY	v
1.0.	**INTRODUCTION**	1
2.0.	**METHODS**	3
2.1.	Mill Reports Examined	3
2.2.	Species Examined	3
2.3.	Tracers Examined	3
2.4.	Chemical Analyses	3
2.5.	Data Analyses	3
2.6.	Conference Session on Pulp Mill Effluent Tracers	4
3.0.	**RESULTS**	5
3.1.	Species-Tracers Interactions	5
3.1.1.	Tracers-Tissues Interactions	5
3.1.2.	Tracers- Analyses Interactions	5
3.2.	Dioxins as Tracers	5
3.3.	Furans as Tracers	6
3.4.	Resin Acids as Tracers	6
3.5.	Fatty Acids as Tracers	7
3.6.	Chlorinated Phenols as Tracers	7
3.7.	Biochemical Tracers	8
4.0.	**DISCUSSION**	9
4.1.	Issues Related to Data Analyses	9
4.1.1.	Detection and Detection Limits	9
4.1.2.	Tracer Presence	9
4.1.3.	Fatty Acids	10
4.1.4.	Inorganic and Biochemical Tracers	10
4.2.	Issues Related to the Working Groups Terms of Reference	10
4.2.1.	Tracer Use in the EEM Program	10
4.2.2.	Difficulties Encountered in Meeting Tracer Requirements	11
4.2.3.	Situations Where Tracers Worked	12
4.2.4.	New Substances as Candidates for Future Tracers	12
4.2.5.	Advantages and Disadvantages of Tracer Use	13
4.2.6.	Issues Related to Tracer Measurement	14
5.0.	**SUMMARY AND CONCLUSIONS**	16
6.0.	**RECOMMENDATIONS OF TRACERS FOR MILLS**	18
6.1.	General Recommendations	18
6.2.	Specific Recommendations	18
6.3.	Recommendations for Further Study	19
7.0.	**ACKNOWLEDGEMENTS**	20
8.0.	**REFERENCES**	21
	TABLES	23
	FIGURES	44
	APPENDICES	50

ABSTRACT

Ali, N., I. Cloutier, S. Humphrey, A. Niimi, R. Parker, C. Wood, and V. Zitko. 1997. Exposed or not exposed: the effectiveness of using tracers to verify exposure of fish to pulp and paper mill effluent. Can. Tech. Rep. Fish. Aquat. Sci. 2162: v + 51 p.

One of the requirements of the cycle 1 environmental effects monitoring program was to conduct an adult fish survey to assess the effects of pulp and paper mill effluents on fish. The use of a tracer was required, as necessary to verify the exposure of fish to the effluent. Chemical tracers, including chlorinated dioxins and furans, resin and fatty acids, and chlorinated phenols, were monitored in 27 species of fish and crab. There were 77 mills that reported tracer observations in fish from the study and reference sites as part of this national program. Some mills used more than one species, and several different chemical tracers that provided 183 data sets from which the effectiveness of using tracers to verify exposure was assessed.

There were a number of technical and operational factors that did not allow a conclusion to be made on effluent exposure. Many of the chemicals that were monitored were below detection, and many of the concentrations measured were close to their detection limits. Some of the difficulties encountered include apparent inconsistencies among the analytical laboratories on the chemical measurements that were reported;in most cases, only a single composite sample from the reference and study site, was used, and little information on chemical concentrations in the effluent was available to compare with that observed in fish. There was also little guidance available on the interpretation of the results. A number of recommendations are included to address these issues, and field validation studies and methodologies are proposed that should provide a better assessment of the effectiveness of tracers in this application.

RÉSUMÉ

Ali, N., I. Cloutier, S. Humphrey, A. Niimi, R. Parker, C. Wood, and V. Zitko. 1997. Exposed or not exposed: the effectiveness of using tracers to verify exposure of fish to pulp and paper mill effluent. Can. Tech. Rep. Fish. Aquat. Sci. 2162: v + 51 p.

Une des exigences du premier cycle du programme de suivi des effets environnementaux a été d'effectuer une étude sur les poissons adultes dans le but d'évaluer les effets d'effluents d'usines de pâtes et papiers sur les poissons. L'utilisation d'un traceur était jugée nécessaire à la vérification de l'exposition du poisson à l'effluent. Des traceurs chimiques, incluant les dioxines et furannes chlorés, les acides gras et résiniques, et les phénols chlorés, furent suivis chez 27 espèces de poissons et de crabes. Soixante-dix-sept usines ont signalé la présence (rapporté des résultats) de traceurs dans des poissons provenant de sites d'étude ou de référence faisant partie de ce programme national. Certaines usines ont utilisé plus d'une espèce de poissons et plusieurs traceurs chimiques, ce qui a foumi 183 groupes de données à partir desquelles on a évalué l'efficacité de l'utilisation de traceurs pour vérifier l'exposition è l'effluent.

Un certain nombre de facteurs techniques et opérationnels ont empêché qu'on pulsse tirer une conclusion quant à l'exposition à l'effluent. Un grand nombre des composés chimiques suivis étaient présents à des concentrations en dassous de la limite de détection, et une grande partie des teneurs mesurées étaient proches de leur limite de détection. Certaines des difficultés rencontrées incluent des désaccords entre les résultats d'analyses chimiques de différents laboratoires, l'utilisation dans la plupart des cas d'un seul échantillon composite du site d'étude et de référence, et le peu d'information disponible surles concentrations de composés chimiques dans l'effluent pour pouvoir fair une comparaison avec celles observées dans les poissons. On a également manqué d'indications suffisantes sur la manière d'interpréter les résultats. On fait des recommandations visant ces questions, et on propose des études et des protocoles de validation sur le terrain qui devraient permettre une meilleur évaluation de l'efficacité des traceurs dans ce domaine d'application.

EXECUTIVE SUMMARY

The objective of the Tracer Working Group (TWG) was to evaluate the Tracer Program, which was designed to supplement the Adult Fish Survey in the first cycle of the pulp and paper EEM. TWG was not involved in the preparation of the program and the selection of tracers.

The rationale behind the Tracer Program was the desire to confirm the exposure of the fish to the effluent, when investigating biological, physiological or biochemical indicators of fish health. The purpose of the Tracer Program was not to measure effects of the effluent on the fish. The chemicals that were primarily used as tracers for the program included chlorinated dibenzodioxins and dibenzofurans, resin acids, fatty acids, and chlorinated phenols.

Chlorinated dibenzodioxins and dibenzofurans (CDDF). From the '2378 class' of these compounds, only the tetrachloro CDF (2378CDF) is a specific potential indicator of exposure to chlorine-using pulp mill effluents, and most mills measuring CDDF found higher levels of 2378CDF in the study zone. The measurements of CDDF are very expensive and, consequently, are not recommended for tracer use.

Resin acids. Four laboratories measured resin acids on behalf of the mills. No intercalibration was performed, and the data provided by the mills, complemented by data from the literature, suggest that systematic differences existed among the laboratories.

For the evaluation of the usefulness of resin acids as tracers, TWG selected nine data sets from 40 mills with prevalently softwood and waste fiber furnish. The selection criteria were based mainly on reports of more than one resin acid present in study zone samples and on resin acids being mostly not detectable in the reference zone. In these selected cases, resin acids could be used to distinguish between fish from reference and study zones. Resin acids are by-products of mills processing softwood and aspen, and cannot be tracers for mills whose furnish is primarily other hardwoods. More work is required in the direction of analytical quality control and assurance, and the fate of resin acids in effluents as a result of various secondary treatment processes.

Fatty acids. Several mills measured 9 fatty acids in the bile or liver of fish. Additional mills measured only oleic acid. No differences could be found between fish and shellfish from reference and study zones. Fatty acids cannot be used as tracers, mainly because of their ubiquitous presence in biological substrates, although it is possible that better results could be achieved by more reliable analyses.

Chlorophenols. *A* paucity of data prevented detailed evaluation of the suitability of using chlorophenols as tracers.

General conclusions. The use of chemical tracers to detect the exposure of fish to an effluent is a good idea which, however, is more difficult to implement, than it may seem. It appears that the Tracer Program was initiated without detailed considerations of many factors. The selection of tracers was reasonable from the current knowledge of compounds in pulp and paper mill effluents. On the other hand, sampling and sample processing protocol have not been clearly established and little attention was paid to chemical analytical factors. As a consequence, the use of tracers in the first cycle of the EEM program was not a success, but provided indications of applicability in principle, and directions for future work. No potential tracers have been identified for hardwood-based mills and further work is needed. It is also necessary to search for potential tracers that survive various forms of secondary treatment of the effluents.

Tracers. The first problem is the choice of chemicals to be used as tracers. These have to be specific for the effluent in question, must be taken up by the fish fairly rapidly, and retained from several days to several weeks in their original or only slightly modified form. Reliable analytical techniques for these compounds must be available and the compounds must be present in concentrations ranging from ng/g (ppb) to μg/g (ppm) to avoid very large errors normally encountered at lower concentrations. Strict quality control and assurance must be implemented.

Samples. Fish or shellfish species must be readily available in the study and reference zones. Sampling protocol, such as the number of specimens taken, their size (age) and sex, and time of the year must be established. Guidelines must be provided for sample storage and processing. It must be decided whether to analyze individual specimens or pooled samples and whether the analyses are to be done singly or in duplicate, and how to deal with unusual values. Sampling and sample processing protocol must be clearly defined.

Recommendations. The TWG recommends that the decision tree provided in Appendix 5 be followed to determine the applicability of fish tracers in subsequent cycles of EEM. A QA/QC program for the measurement of tracers in samples of fish tissue is required. Field validation studies should be initiated prior to, or in conjunction with, the cycle 2 program, to assess the effectiveness of the revised tracer protocol.

1.0 INTRODUCTION

In May, 1992, the Federal Government of Canada passed amendments to the Pulp and Paper Effluent Regulations (PPER) under the authority of the *Fisheries Act*. The amendments included revised limits on biochemical oxygen demand, total suspended solids and acute toxicity to fish which were more stringent than those contained in the 1971 regulations. As well, the amended regulations applied to every pulp and paper mill in Canada. There was provision for a transitional period to allow all mills to make any modifications or improvements necessary to meet the new effluent quality limitations. All mills were obligated to be in compliance with the amended regulations by December 31, 1995.

In addition to the changes in effluent limits, the amended PPER required every mill to conduct an environmental effects monitoring (EEM) program. The objective of the EEM program was to determine if the fish, the fish habitat and the utilization of the fisheries resource were being adequately protected by the requirements of the PPER. The EEM studies were to be conducted on three year cycles with the first reports due on April 1, 1996. The requirements for the environmental monitoring studies were set out in Sections 28 - 35 of the PPER, in a supporting requirements document (AEEMR.1992a) and in Annex 1 (AEEMR. 1992b) of the requirements document.

All mills were required to conduct an adult fish survey, a benthic invertebrate survey, sub-lethal toxicity tests on effluent, measurements of chlorinated dioxins and furans in fish tissue if the mill had used chlorine, and some supporting water and sediment quality measurements. As well, a fish tainting study was required if there was a well documented complaint about tainting.

In the adult fish survey (AFS) component of the EEM study, Annex 1 directed that an effluent tracer be measured in the fish "... to substantiate exposure of fish to the liquid phase of the effluent in the receiving environment for the fish survey." The Annex recommended that compounds such as chlorinated dioxins and furans, resin acids, fatty acids and chlorinated phenols all had potential for use as effluent tracers that would accumulate in fish. Limited technical guidance about tissue types, sampling procedures, sample sizes and analytical methods were provided in the supporting documents of the EEM program.

During the first EEM cycle, a number of issues were raised about the fish tracer requirement. Identifying suitable effluent tracers, particularly in secondary treated effluent, was problematic. The concentrations of resin and fatty acids and many of the chlorophenols were dramatically reduced by secondary treatment or the reduction in the use of elemental chlorine in bleaching. For resin and fatty acids, bile was identified as the tissue that should contain the maximum concentration of these compounds. Collecting sufficient quantities of bile, particularly from smaller fish, created many challenges. There were only a few commercial laboratories particularly at the start of the program that were experienced with measuring low levels of these tracer compounds in fish tissue. As the need for these types of analyses increased, several other laboratories began to offer the analyses but standard analytical methods and standard reference materials for some tracers were not available. There were no interlaboratory comparisons of analytical results. As the first cycle EEM reports were being reviewed, it became obvious that very few studies had conducted simultaneous sampling of effluent, receiving water and fish tissue for the selected tracers. This made compilation and interpretation of the tracer results difficult.

As a result of these and other problems with the use of fish tracers in the first EEM cycle, a Tracer Working Group was formed in December, 1995. The original eight member group was made up of Environment Canada regional EEM staff, research scientists from the Department of Fisheries and Oceans and representatives from the pulp and paper industry. Group members included:

Nardia Ali. Environment Canada, Downsview, ON, M3H 5T4;
Isabelle Cloutier. Abitibi-Price Inc., Quebec, QC, G1R 4S1;
Susan Humphrey. Environment Canada, Downs-view, ON, M3H 5T4;
Arthur Niimi. Department of Fisheries and Oceans, Burlington, ON, L7R 4A6;
Roy Parker. Environment Canada, Dartmouth, NS, B2Y 2NY;
Craig Wood. Noranda Inc., Pointe-Claire, QC, H9R 1G5;
Vladimir Zitko. Department of Fisheries and Oceans, St. Andrews, NB, E0G 2X0; and
Raymond Van Coillie. Environment Canada, Montreal, QC. (Resigned September, 1996).

The Working Group operated by having bi-weekly teleconferences and occasional face-to-face meetings. There were 24 teleconferences and 6 meetings held during the preparation of this Report.

The objective of the Working Group was to evaluate the purpose and effectiveness of using pulp and paper effluent tracers in fish. The group examined the tracer requirement of the EEM program requirements for cycle 1 and developed the following statement: "The purpose for requiring Canadian pulp and paper mills to analyze for tracers in fish during the adult fish survey, at mill sites where fish can move freely between sampling locations, is to verify that the fish collected in the receiving area have been exposed to the effluent, and that the fish from the reference area have not."

The Terms of Reference developed by the Working Group were to:

[1] review the concept of effluent tracer use in the EEM program;
[2] summarize difficulties encountered by mills required to measure an effluent tracer in fish;
[3] document situations where measurement of effluent tracers in fish was not problematic;
[4] identify substances present in secondary treated effluent as well as substances that can safely be added to effluent which might be candidate tracers for future EEM studies;
[5] assess advantages and disadvantages for use of tracers in intake water, treatment system, outflow and receiving waters based on literature, ongoing research, available data;
[6] identify issues relating to tracer measurement including information on availability of commercial analyses; and
[7] recommend some tracers that might be appropriate for different pulp and papermaking processes, marine and freshwater environments, shellfish and finfish, and document the strengths and weaknesses of each.

The complete terms of reference for the Tracer Working Group is found in Appendix 1.

This Tracer Working Group Final Report will be provided to the joint government-industry EEM Technical Management Committee so that the conclusions and recommendations can be considered by that committee as they determine the requirements for the next cycle of EEM studies at Canadian pulp and paper mills.

2.0. METHODS

The Working Group decided early in its discussions that tracer related data from all mill reports submitted by April 1, 1996, should be examined in detail to gain a national perspective on the issues. A contractor, I. Menard, Montreal, QC, was assigned the task of compiling data from the mills. Other members of the Working Group also compiled data to examine specific issues.

2.1. MILL REPORTS EXAMINED

There were 134 mills that participated in the EEM program across Canada. All mills were assigned an alphanumeric code for identification (Appendix 2). Among these, 77 mills reported tracer chemical data in support of the adult fish survey by April 1, 1996. Some of the remaining 57 mills not included were exempted from this requirement because of barriers such as dams that separated the study and reference sites, or local conditions that did not allow samples to be collected in a manner that would be suitable for the intended purpose. New mills were not considered because their reports were due after April 1, 1996. Joint reports were submitted by 17 mills because effluents were discharged into common receiving waters and contributions from individual mills could not be clearly separated. These included five 2-mill reports, one 3-mill report, and one 4-mill report.

The Working Group assessment was based on data in 67 *mill reports*, that included individual and multi-mill reports. Some mill reports included data on more than one species, and each is reported as a *data set*. There were 183 data sets examined by the Working Group that largely formed the basis of this Report.

2.2. SPECIES EXAMINED

The large number of species used in support of the adult fish survey can be attributed to the geographic diversity of the effluent receiving waters. There were 27 species that were used among the 67 mill reports (Table 1). These represented 15 freshwater and 9 saltwater fish species, and 3 species of crabs.

2.3. TRACERS EXAMINED

There were 100 chemicals that were monitored as tracers, including 92 organic, 7 inorganic chemicals and 1 biochemical (Table 2). Organic chemicals included 10 chlorinated dibenzo-*p*-dioxins (DD - referred to as dioxins in this report), 12 chlorinated dibenzofurans (DF - referred to as furans in this report), 12 resin acids (RA), 13 fatty acids (FA), and a general group of chlorinated phenols (CP) that included 16 chlorophenols, 11 chloro-guaiacols, 8 chlorocatechols, 3 chloroveratroles, and 7 other compounds. Inorganic chemicals included copper, iron, manganese, mercury, phosphorus, strontium and zinc. Ethoxyresorufin *O*-deethylase (EROD) was the only biochemical tracer used.

2.4. CHEMICAL ANALYSES

Six analytical laboratories conducted all the organic chemical tracer analyses reported in the 67 mill reports (Appendix 3). These included Canviro Analytical Laboratories, Waterloo, ON; Enviro-Test Laboratories, Edmonton, AB; Fenwick Laboratories, Halifax, NS; Novamann International, Montreal, QC and Toronto, ON; Wellington Laboratories, Guelph, ON; and Zenon Environmental Laboratories, Montreal, QC, Burlington, ON and Burnaby, BC.

2.5. DATA ANALYSES

Three approaches were used to assess tracer issues because of the large number of mill reports and diversity among the information reported. These included 1) comparisons of ratios in tracer concentrations between reference and study sites, 2) analyses on the range of concentrations for different chemicals, and 3) Principal Component Analysis (PCA).

1) A review of the tracer data reported in the mill studies indicated most tracer concentrations were based on a single composite sample each from the study and reference sites. To evaluate the suitability of a chemical as a tracer, the concentrations in fish from the study site were divided by the concentrations in fish from the corresponding reference site. If the latter was 0 or not reported it was arbitrarily set to 0.1 of the respective unit. Some studies reported samples that were taken at several points in the study area. The site closest to the mill discharge was used in these cases.

   Data reported by some mills, particularly for resin and fatty acids, included a detectable concentration at one site while the other site reported nondetectable levels whose detection limit was higher than the concentration that was detected. This anomaly was largely due to insufficient sample weight of bile for analyses. In this report non-detectable concentrations were reported for both sites in these cases.

2) The second approach involved the use of comparisons between reference and study sites to assess the differences between sites. It was based on their average concentrations, and differences between concentrations by factors of 2, 5, 10 and 100 fold.

   These comparisons were sometimes arbitrary because some chemicals were below detection limits, and in these cases the detection limit was used for calculation purposes. Comparisons between sites examined the range of tracer concentrations that were reported for some species and tissues to assess this variability on a national basis. This approach was used to gain an insight into the reliability of drawing conclusions on the level of tracer exposure from the data reported in support of the adult fish survey.

3) PCA was used to analyze the multivariate data on 9 resin and 9 fatty acids. PCA projects the original nine-dimensional spaces on spaces with fewer dimensions in a way that retains most of the original information (Malinowski, 1991; Zitko, 1994). The positions of the samples are expressed in new "principal components" coordinates, and are linear combinations of the original coordinates (variables). The result is "scores" plots, in which the similarity of samples is inversely proportional to their distance, and "loadings" plots which show the influence of the original variables on the principal components. In the loading plots, the distances of the variables are inversely proportional to their correlations.

2.6. CONFERENCE SESSION ON PULP MILL EFFLUENT TRACERS

The Tracer Working Group organized a special half-day session at the 23rd Annual Aquatic Toxicity Workshop to discuss the preliminary findings of the group and to provide an opportunity for other researchers to contribute to the group's project. The session consisted of a few platform presentations and posters and a general discussion session. A summary of the tracer session is provided in Appendix 4.

3.0 RESULTS

3.1. SPECIES-TRACERS INTERACTIONS

White sucker was used in 29 of 67 mill reports, and was the most common species used for monitoring each organic chemical group (Table 3). Longnose sucker, winter flounder and walleye were used in 7 or 8 reports. There were 8 species used in 2 or 4 reports, and 15 species used in 1 report.

3.1.1. Tracers-Tissues Interactions

Tissues that were monitored for organic chemicals included muscle, liver, bile, liver/gall bladder and hepatopancreas. Limited guidance regarding fish tissue to be sampled for tracer measurement was provided for the first EEM cycle.

Muscle was the only tissue monitored in the 34 data sets for dioxins and furans (Table 4). Bile was used in about 60% of the analyses for resin and fatty acids, while liver, liver/gall bladder, and the hepatopancreas were used in a few studies. Liver was the most common tissue for chlorinated phenols.

3.1.2. Tracers-Analyses Interactions

The dioxin and furan measurements were conducted on the same sample of fish muscle and were analyzed simultaneously by one of the laboratories identified in Appendix 2. Analyses for resin and fatty acids in bile were generally conducted by the same laboratory, although some studies did not measure fatty acids. In general, the analyses of each chemical group were conducted by 3-5 laboratories (Table 5). No single laboratory analyzed more than 40% of dioxin, furan and resin acid analyses, but 3 laboratories were major contributors to these chemical groups.

3.2. DIOXINS AS TRACERS

There were 29 mill studies that reported dioxins in the muscle of 13 fish and crab species. From these, 34 data sets were assessed because several mills monitored more than 1 species. Two mills, AB6 and AB7, also reported DD 100, 101 and 102 which are Cl_2 and Cl_3 congeners, but these were below detection limits at the reference and study sites for both mills. These congeners were not included in the dioxin data summary analyses. A total of 238 measurements of Cl_4-Cl_8 congeners in fish tissue from both reference and study sites were evaluated by the Tracer Working Group.

Among the 34 data sets, 15 have no detectable concentrations in samples from either site (Table 6). At least one congener was detected in 19 data sets, and a total of 27 congeners were detected in samples from reference sites, and 34 congeners from the study sites. There were 5 data sets with the same number of congeners detected at both sites, 4 had more congeners at reference than study sites, and 9 had more at the study sites.

Comparisons among congener concentrations indicated 6 data sets had a greater number of congeners with higher concentrations at the reference than study sites, 11 had more at the study sites, and 2 had the same number for both sites. Further analyses indicated 3 data sets had more congeners with concentrations that were 2X greater at the reference sites, and 6 had more at the study sites. One reference site sample had more congener concentrations that were 5X higher than at the study site. There were no samples where the differences between study and reference sites were greater than 10 fold. There were 12 data sets where the same 1-3 congeners were detected at both sites.

2,3,7,8-TCDD (DD1) was detected at 1 reference site and 6 study sites while DD3, 4 and 5 were not detected in any samples. The hepta- and octachloro- congeners (DD6 and 7) were present in over 50% of the 19 data sets at both sites (Table 7). Comparisons among all detectable dioxin concentrations for all data sets indicate little or no differences between study and reference sites. A similar conclusion was indicated when the same congeners found in samples from both sites were also compared.

3.3 FURANS AS TRACERS

Furans were not detectable at either site for 9 of the 34 data sets (Table 8). There were 25 data sets where at least one congener was detected, a total of 30 congeners were detected in reference sites, and 37 at the study sites. There were 8 data sets with the same number of congeners detected at both sites, 5 had more congeners in the reference sites than study sites, and 12 had more congeners at the study sites. With one exception, DF10 was present in higher concentration at the study sites.

Comparisons among congener concentrations indicated 6 data sets had a greater number of congeners with higher concentrations at the reference sites than study sites, 19 data sets had concentrations greater at the study sites than the reference sites, and 1 data set had the same number for both sites. There were 3 data sets which had congeners with concentrations that were two times greater in reference site than study site. Another 14 data sets had concentrations of furan congeners that were two times higher at the study site than at the reference site. There were 5 data sets where the furan congener concentrations were five times greater in the study site samples than the reference site, and at 3 study sites, congener concentrations at study sites were 10 times higher than the reference sites. Among the 16 data sets where furans were detected at both reference and study sites, 15 had 1-3 congeners that were common to both.

The furan concentrations which were detected in the 25 data sets indicated; 2,3,7,8-TCDF (DF10) was present in 13 data sets samples from reference sites and 21 data sets from study sites (Table 9). DF11, 12 13, 14, 15, 16, 17 and 19 were detected in a few samples, while DF 18 was not detected at all. Comparisons among all detectable concentrations indicated concentrations of DF10 were generally higher at the study sites than reference sites. There were 10 fish species that were represented among the 21 data sets where DF10 was detected at the study site. Of these, 20 of 21 concentrations were higher at the study than the reference site.

3.4. RESIN ACIDS AS TRACERS

Resin acids were the most common chemical group measured as tracers. There were 48 mill studies that reported resin acids in 18 species of fish and crabs, and 63 data sets were examined (Table 10, 11). About 8-11 resin acids were monitored by most studies. Bile was analyzed in 39 data sets, other tissues analyzed less frequently included liver, liver/gall bladder, muscle and hepatopancreas. Concentrations of resin acids are summarized in Table 10. Resin acid data reported by mills AB6 and AB7 for whitefish were based on the same reference sample, only one data set was used. In contrast, dehyroabietic acid (RA21) concentrations of 110 mg/kg in sucker reported by mill ON21 appeared high, but concentrations of other resin acids appear consistent with other studies; therefore, all values for this data set were used.

A total of 600 resin acid measurements were conducted at both reference and study sites. There were 95 resin acids detected at reference sites, and 149 at study sites. There were 12 data sets with no detectable levels at either site, and 11 data sets with the same number detected at both sites. A higher number of resin acids were detected at the reference site than study site in 11 data sets, and there were 29 data sets where more were detected at study sites. Comparisons between sites indicated 8 data sets had more mean concentrations that were greater at reference sites than study sites, and 18 had more at the study sites, while 13 had the same number between sites. Furthermore, 5 data sets had concentration differences that were 2 fold higher at reference than study sites, and 14 were higher at the study sites. Comparisons based on 5 fold differences indicated 7 data sets were higher at reference sites, and 11 were higher at study sites. There were 5 data sets that were higher at reference sites based on a 10 fold difference, and 12 were higher at study sites. One data set had a concentration difference of 100 fold at the reference site, while 4 have the same difference at study sites. There were 32 data sets that included 63 resin acids that were common to the study sites and the reference sites.

As can be seen from Table 12, dehydroabietic acid (RA21), isoprimaric acid (RA22), and abietic acid (RA20), were most frequently detected and were most consistently present in higher concentrations in fish from study sites than from reference sites.

PCA projections show a separation between reference (R) and study (S) sites (Fig. 1). The separation occurs mainly along the pc-1 axis. Projections on other pc planes show similarities and differences of resin acid profiles. These have a potential to become another useful indicator (Fig. 2). The loading plots (Fig. 3, 4) show the relationships among the resin acids and their influence on the individual principal components and also have a further diagnostic potential.

3.5. FATTY ACIDS AS TRACERS

There were 28 mill studies that reported fatty acids in 14 species of fish and crab, and 38 data sets were assessed (Table 13). The number of fatty acids reported varied widely, 17 data sets reported 2 fatty acids, and 20 data sets reported 9-11. Bile was analyzed in 21 data sets, and other tissues analyzed included liver, liver/gall bladder and hepatopancreas.

A total of 240 fatty acids were measured in 38 data sets for samples collected from both the reference and study sites. There were 32 data sets where the same number of fatty acids were detected at both sites. There were 6 data sets where more fatty acids were detected at reference than study sites, but no data sets where more fatty acids were detected at the study sites. Comparisons of differences in fatty acid concentrations between sites indicated that 19 data sets had concentrations that were higher at the reference sites, and 17 had higher concentrations at the study sites. Comparisons based on 2 fold differences indicated that 12 data sets had concentrations higher at reference sites, and 7 at the study sites. Comparisons based on 5 fold differences indicated 7 data sets were higher at reference sites, and 5 data sets at study sites. Comparisons at the 10 and 100 fold level of differences also indicated similar trends where more concentrations were higher at the reference sites relative to the study sites. Of the 38 data sets examined, there were 36 where the same fatty acids were detected at both sites.

Many of the fatty acids detected were at mg/kg concentrations in a large number of data sets (Table 14). The following fatty acids, 9,10-Dichlorostearic acid (FA51), and palmitoleic acid (FA52), were not included in the results because levels were below detection limits in the few studies where they were measured. Lignoceric acid (FA45) was detected at 2 reference sites at 0.4 mg/kg, and 1 study site at 0.5 mg/kg in bile of sucker at mills QC12 and QC17.

As can be seen from Table 15, there was rarely a considerable difference between fatty acid concen-tration and study sites and the corresponding reference sites.

Several mills measured 9 fatty acids in the liver and bile of tomcod and mummichog, in the hepatopancreas of green crab in the marine environment, and, in fresh water, in the bile of white sucker. For the first three species, there is no separation between samples from reference (R) and study (S) areas in any of the PCA projections (Fig. 5-9).

3.6. CHLORINATED PHENOLS AS TRACERS

The chlorophenols, chloroguaiacols, chloro-catechols, chloroveratroles, chlorosyringol, and several other chemicals (Table 2) were collectively assessed as a group because they were often measured together. There were 9 mill studies that reported chlorinated phenols in 11 species of fish and crabs (Table 16). Liver and bile were the most common tissues analyzed, with muscle and hepatopancreas examined in a few studies. There were 15 data sets reported, although only 14 were assessed because the values for white sucker reported by mills QC31 and QC49 were identical.

There were 384 chlorinated phenols measurements that were conducted among the 13 data sets, 13 chemicals were detected at the reference sites and 20 at the study sites. Comparisons of their concentration differences between sites indicated 7 data sets have more mean concentrations that were higher than reference sites, and 3 data sets sites where the reference sites were higher than the study sites. These comparisons are of limited significance because of the low number of chemicals detected compared to the other organic chemical groups examined.

There were 8 chemicals represented among the 13 detectable concentrations reported at 5 reference sites (Table 17). 3,4,5-Trichloroguaiacol (CP74) and 4,5,6-trichloroguaiacol (CP76) were the most common and detected at 3 reference sites. Five chemicals were detected only at one reference site each. There were 12 chemicals represented among the 21 detectable concentrations that were reported at 10 study sites. 4,5-Dichloroguaiacol (CP72) was detected at 4 study sites, and 3,4,5-trichlorocatechol (CP83) was detected at 3 study sites. There were 7 chemicals detected at one study site each.

3.7. BIOCHEMICAL TRACERS

Activity levels of ethoxyresorufin O-deethylase (EROD), one of mixed function oxygenases, were used in 3 mill studies (Table 18). Activity levels among the 4 fish species examined were generally in the 2-10 pmol/min/mg protein range. No differences in activity levels were suggested for most mills, and those where differences were suggested are based on small sample sizes. A mean activity level of 49 pmol/min/mg protein for whitefish reported by mill AB7 was based on 3 values below detection limits and values of 52 and 190 pmol/min/mg protein; no values were reported for fish from the reference site in this study.

4.0. DISCUSSION

4.1. ISSUES RELATED TO DATA ANALYSES

4.1.1. Detection and Detection Limits

Information that was presented in the results section focused primarily on tracers that were detected. Only 3-5% of all the chlorinated phenols measured at the study and reference sites were detected, 9-11% of chlorinated furans, 11-14% of chlorinated dioxins, and 16-25% of resin acids. Fatty acids were detected at 68-71% frequencies at both study and reference sites (Table 19).

Four laboratories measured resin acids on behalf of the mills. Since there was no intercalibration, it was of interest to compare their data with that reported in the literature for resin acids in the bile of trout, roach and perch (Oikari, 1986; Oikari and Holmbom, 1986; Oikari et al., 1980, 1982, 1984). To include the largest number of literature data, only four resin acids - pimaric, isopimaric, abietic and dehydroabietic acid were considered in this comparison.

In the PCA projections (Fig. 10, 11), one would expect a random distribution of data points ('x' - literature, 1, 3, 5, 6 - laboratories). This is not the case and the data suggest systematic differences among the laboratories.

The combined factors of a low percentage of tracers detected, and concentrations near their detection levels were important issues in assessing the effectiveness of tracers. As can be seen from Table 7, the most consistently detectable dioxins were DD6 and DD7. However, the concentrations of these dioxins were similar at both the reference and study sites.

Similarly from Table 9, one can see that only DF10 was consistently detectable in both the study and reference sites. The concentrations in the study site with one exception were higher than those found in the reference site.

4.1.2. Tracer Presence

A decision to monitor tracers at a specific mill would be based on the confirmation of their presence in mill effluents. There were 48 mill studies that monitored resin acids as tracers; this decision would be supported by various studies that have indicated wood is an important source for naturally occurring compounds such as resin and fatty acids, and some phenols (Plam et al., 1995). Softwoods are generally considered important sources of resin acids compared to the other furnish used by mills. There should be no resin acids in hardwoods with the exception of aspen which is known to have extremely high levels of wood extractives (Chen et al. 1994). An analysis on the furnish data available on 44 of the 48 mills indicated about 66% reported using primary softwood furnish (Table 21). There were 19 mills where furnish was 100% softwood, and 10 mills that used at least 80% softwood furnish. Among these 29 mills, 13 had secondary treatment. The significance of treatment process on mill effluents was not examined by the Working Group because of limited information on resin acid concentrations in effluents and effluent mixing zones. Secondary treatment has been reported to significantly reduce (>80%) the concentrations of resin acids in final effluent (Voss 1987).

Dehydroabietic and abietic acids were the two most common tracers identified based on their presence in white sucker (Table 12). A tabulation of the concentrations that were reported in effluent, and study zone, and reference zone for comparison, would suggest a relationship with the percent of softwood furnish (Tables 20-22). There were 15 studies where dehydroabietic acid was measured at the study sites, and 10 were at or below the 2 µg/L detection limit. The same trend was shown for 4 of the 7 studies that monitored abietic acid.

A number of mills included several reference and study sites in the adult fish survey to assess near-field, far-field and far-far-field effects (e.g. ON4, AB5). An evaluation of the tracer data generally indicated

inconsistent trends in trying to relate their concentrations to the proximity of the sample site to the mill source. This might be attributed to several factors including tracer concentrations too near background levels.

4.1.3. Fatty Acids

Fatty acids in fish were detected in 36 of the 38 data sets examined, and 32 data sets detected the same number at both sites (Table 14).

About 70% of all fatty acids measured were detected. (Table 13) Fatty acids would not be suitable as a tracer because they are commonly present in biological substrates. Fatty acid concentrations in organisms would be influenced by homeostatic processes because they are essential products. Fatty acids were also the only organic chemical tracer examined whose concentrations tended to be higher at the reference than study sites (Table 14, 15).

4.1.4. Inorganic and Biochemical Tracers

The small data set for inorganic chemicals was not sufficient to evaluate their effectiveness as tracers. Only mercury and strontium can be viewed as xenobiotic substances among those monitored. The other chemicals, including sodium that can be present in high concentrations in some effluents, may not serve as an effective tracer because their concentrations in organisms are regulated. Little information is available in the literature to assess the effectiveness of inorganic chemicals as tracers in this context.

EROD induction does not appear to be an effective tracer based on the limited data presented (Table 18). Activity levels between study and reference sites appeared to be similar. This might be attributed to the low concentrations of mixed function oxygenases inducing chemicals in the effluent. Chemicals such as the 2,3,7,8-substituted chlorinated dioxins and furans are potent inducers, but the level of induction reported here is substantially less than that induced in the laboratory in fish exposed to higher concentrations (e.g. van der Weiden et al., 1994). Laboratory studies have reported resin acids are poor EROD inducers (Ferguson et al. 1992). The low level of induction reported in the mill reports might be attributed to the relatively low levels of dioxins and furans that were reported in various mill studies (van der Weiden et al. 1994). Other research has indicated that dioxins and furans are not the only EROD inducers present in pulp mill effluents. EROD was the only biochemical tracer examined by some mills; therefore, the effectiveness of other biochemical tracers cannot be discounted at this time. Some biochemical tracers such as EROD may have a limited use as tracers because they can be induced by a number of chemicals. It could be difficult to identify the specific chemical in an effluent that would be the primary inducer.

4.2. ISSUES RELATED TO THE WORKING GROUP'S TERMS OF REFERENCE

4.2.1. Tracer Use in the EEM Program

Methods that were used by mills to meet the tracer requirement were largely based on information described in various technical guidance documents. Tracer chemicals monitored by mills included Cl_4-Cl_8 dioxins and furans, resin and fatty acids, and chlorinated phenols. Selection of these chemicals may be attributable to two factors. Section 9.1 of the AEEMR (1992b) stated all mills that had used chlorine as part of their bleaching process were required to monitor all 2,3,7,8-substituted chlorinated dioxins and furans in the edible portion of fish and other aquatic organisms. This requirement was done to address human health concerns, although some mills appeared to use this data also to meet their tracer requirement. Section 11.0 of the same document also identified chloroguaiacols, chlorocatechols and dehydroabietic acid as chemicals that could be present in the water column for measurement, and "it will likely be necessary to select a tracer which will be accumulated in fish tissue." The basis for selecting fatty acids was less clear because they did not appear to be specifically identified in the technical documents as potential tracers, but may have been included because they were also listed along with resin acids in most of the analytical methods sections.

Dioxin and furan measurements in fish were conducted on muscle samples, while liver, bile and hepatopancreas were used for other chemical groups following the guidance provided in Section 12.1 of the TGD (1993). The analytical methods, and quality assurance/quality control measures, used for chlorinated dioxins and furans, resin and fatty acids, and chlorinated phenols were described in Table 6.2 of TGD (1993).

The issue of sample size was less clear. Section 12 of the TGD (1993), described the tissues to be sampled, but did not specify the number of samples to be examined. Section 5.5 of the same document stated "six samples per species and tissue type are the recommended level of effort" for mills that were required to monitor for chlorinated dioxins and furans in fish, molluscs and crustaceans. Section 9.1 of the AEEMR (1992b), stated that "For the first EEM cycle, analyses will be conducted on a composite sample of 10 individuals of a single species for the near-field area and reference area" as part of chlorinated dioxins and furans requirement. It might be assumed that the strategy of one composite sample from each site was also applied to the other organic chemical groups by a majority of studies.

Detailed explanations on the rationale, method of evaluation, and basis for deriving conclusions were presented in several technical documents for many requirements of the EEM program. Similar guidance was not provided for tracers.

In cases where there are no suitable compounds in the effluent that could serve as a tracer in fish, a mill could use a water column tracer approach. There are a broader range of compounds or measurements (e.g. conductivity, colour, sodium) that can be measured in the receiving waters to confirm and quantify the presence of the mill effluent. This approach could at least confirm that the fish were captured in an area where there was mill effluent present. The weakness of this approach lies in not knowing anything about the duration of fish exposure to the effluent.

4.2.2. Difficulties Encountered in Meeting the Tracer Requirements

A tracer is useful only if it can be detected. Requirements for the adult fish survey specified that samples be collected in the 1.0% effluent mixing zone (AEEMR, 1992b). This criterion was the contributing factor to the relatively low tracer concentrations reported in fish. The 1% mixing zones at most mill sites were generally defined using a fluorescent dye such as Rhodamine WT. Characterization of this zone was done prior to the adult fish survey in most cases. This zone is generally defined on the basis of other parameters rather than the tracers themselves. It is probable that the concentrations of the tracers in the 1% zone could be sufficiently low that their levels in fish are near or below analytical detection limits.

Few mill studies reported effluent concentrations in water that were collected during the adult fish survey. Effluent concentrations of <0.08% at mill ON17 and 0.01-0.05% at mill AB3 were reported. Variations of this magnitude were because of the difficulties in defining the 1% effluent mixing zone.

Discharge into a high flow river would dilute the effluent to 1% within 25 m of the diffuser during average flows (mill ON22). In contrast, discharge into a low flow river would result in the 1% effluent plume occurring 80 km downstream under average flow, and 300 km downstream under low flow conditions where it enters a large water body (mill ON3). Some hydrological conditions may also restrict the extent of the 1% effluent zone to only one side of a river (mill SK1). Selection of a sentinel species can also influence the effectiveness of tracers because tracers are used in support of the adult fish survey, and not the opposite. Species selection is determined by availability, and factors such as low mobility and prolonged residency time to the effluent exposure are important (FG, 1995). Selection of a reference site that replicates conditions at the study site was another criterion for selecting the species to be monitored. The consequences of selecting a sentinel species in a section of the effluent receiving area whose habitat is comparable to the reference site may not always coincide with the prescribed 1% mixing zone.

The use of tracers was based on the premise that chemicals discharged in mill effluent would be accumulated by fish that frequent these waters at higher concentrations than fish from other waters. Major

issues included the detection of tracers as noted previously, reliability of the levels detected, and confidence in interpreting the data.

Several issues must be resolved before developing a decision making process based on tracers. A decision based on statistical analyses would be preferred although not likely because the low sample numbers reported do not allow an estimate of variance. Comparisons based on differences in mean concentrations of composite samples from each site would also require criteria on data interpretation to be established. These criteria must also consider the likely consequences of dealing with a varying number of tracers, and their presence and/or absence at the reference site. Another issue to be resolved is clarification on the significance of tracers found in fish from the reference site. Resin acids, fatty acids, phenols, and other natural chemicals are found in wood products; therefore, their ubiquitous distribution in fish from reference sites would not be unexpected. Chlorinated dioxins and furans are industrial by-products and can be distributed by long range atmospheric transport; therefore, their presence in fish from reference sites could be expected.

Many of the tracers occur as groups of related compounds. The profiles (proportions of components) in such mixtures may contribute additional information and be used for diagnostic purposes. Multivariate mathematical techniques such as the Principal Component Analysis (PCA) may be used to study the profiles.

Many of the tracers detected in fish from the study sites were also detected at the reference sites. Most of the fish collected for the adult fish survey at both sites were captured within a period of a few days to several weeks. Distances between reference and study sites varied considerably. In addition, at many mills, it would have been possible for the fish to move freely between the study and reference sites. This could explain the observations where reference site fish had higher concentrations of tracers than fish from the study site. It was previously noted that chlorinated phenols concentrations in mill AB6 appeared higher at the study site. Yet fish were collected one year apart, and reference and study sites were more than 250 km apart. Issues like this draw attention to the need for guidance in the application of tracers, and the broader issue of the adult fish survey.

4.2.3. Situations Where Tracers Worked

From the '2378 class' of these compounds, only the tetrachloro CDF (2378CDF, DF 10, Table 2) is a potential indicator of exposure, and most mills measuring dioxins and furans found higher concentrations of DF10 in the study zone.

Resin acids as tracers worked well in nine mills selected by TWG from forty mills with prevalently softwood and waste fiber furnish. The selection criteria were based mainly on the reports of more than one resin acid and resin acids mostly not detectable in the reference zone. Of the nine selected mills, five have primary clarifiers, two have aerated stabilization basins, one has screening, and the effluent treatment of the remaining mill is unknown at the moment.

The resin acid profiles may carry additional information in respect to furnish, bleaching, treatment, and fish species, but the data are insufficient for any conclusions. It can only be noted that the three 'outlying' clusters come from study areas of three mills with different processes and treatment. PCA also readily draws attention to the unusually high level of sandaracopimaric acid in one sample.

4.2.4. New Substances as Candidates for Future Tracers

A review of the literature has not identified any additional organic chemicals potentially useful as tracers. It is possible that research in the field of wood extractives may yield some potential tracers. Chlorinated phenols appeared to be the most promising group for future studies, but most were below detection limits. Previous laboratory and field studies suggest higher chlorinated guaiacols could be potential tracers because they have been found in fish and sediments of pulp mill effluent receiving waters, and are moderately persistent in fish. But their role as tracers was not supported by the mill studies examined by the Working Group (Niimi et al., 1990; Trapp et al., 1994).

A review of the literature indicated a large number of chemicals have been identified in pulp and paper mill effluents, sediments, biota and receiving waters (e.g. Suntio et al., 1988; Healey et al., 1994). More recent studies on specific chemical groups include the chlorocymenes, chloro-phenanthrenes and chlorodibenzothiophenes (Koistinen et al., 1994; Sinkkonen et al., 1994; Rantio and Paasivirta, 1996). Many of the chemicals reported in these studies were natural compounds associated with wood products.

The fate of resin acids during various secondary treatments of the effluents has to be determined. Some products formed from resin acid by secondary
treatments may be potential tracers (Zender et al., 1994; Tavendale et al.; 1996; Zhang et al. 1997).

Thus far, no potential tracers have been identified for hardwood-based mills. There is a need to look at concentrations and fate of other relatively hydrophobic compounds, resistant to rapid biodegradation, in the effluents. Plant sterols (Cook et al., 1997) may yield potential tracers. This is a long-term research proposition.

The addition of a chemical during mill operations could serve as a tracer. A unique substance can be added under controlled conditions to process water to achieve a more stable and predictable waterborne concentration at the study site. The same chemical, or those with similar properties, could also be used by a large number of mills and some degree of standardization in the use and interpretation of tracer data could be developed. A substance unique to the mill could resolve difficulties of fish being exposed to effluents from other mills in close proximity.

There are a number of disadvantages to adding a synthetic or naturally occurring chemical tracer. Major issues could include regulatory concerns, costs, developing the necessary information on its physical and chemical properties in diverse aquatic environments, and its potential biological and toxicological effects on organisms. Regulatory statutes such as the Fisheries Act Section 36 (3) states "... no person shall deposit or permit the deposit of a deleterious substance of any type in waters frequented by fish or in any place under any conditions where a deleterious substance that results from the deposit of the deleterious substance may enter any such waters." Provincial and regional authorities may have similar legislated requirements.

The amount of tracer required could be high, up to 10kg may be needed each day. This estimate is based on a 1 cubic meter per second rate of effluent discharge, and a projected concentration of 100 μg/L at the outfall, so waterborne concentration in the 1% mixing zone would be 1 μg/L. This quantity could be increased by a factor of 10 or more if higher waterborne concentrations are required to provide concentrations in fish well above the analytical detection limits, or lower if analytical detection limits in water and tissues are lower. Many other physical, chemical and biological factors that could affect the stability of the tracer in aquatic ecosystems must also be considered.

The tracer itself should have no deleterious effects on fish, and should only serve its intended purpose. It should also not act as a chronic or acute toxicant, or a stimulant, to other organisms including invertebrates and aquatic plants because of its implications on habitat assessment. Extensive laboratory and field studies would be required to address these and other complex issues.

4.2.5. Advantages and Disadvantages of Tracer Use

There are clear advantages of using tracers or other markers to verify that the sample fish collected for the adult fish survey would be representative of the population that inhabit the effluent receiving area. There are advantages of using tracers to indicate fish from the reference site were not exposed to a particular mill effluent, but suffice it to note that this may not always be used as the basis for resolving cause-and-effect responses relating to fish health between study and reference sites because of other issues.

Tracers may be used to indicate that fish were exposed to mill effluents, but the unrestricted movement of fish would preclude further conclusions on the duration of exposure being made. Laboratory studies on the kinetics of chemicals in fish have provided a large body of information that can be applied to assess issues

such as this. Contaminant dynamics models may provide an estimate on how long an organism has been exposed to a chemical; however, these are based on the premise of near steady-state exposure conditions, or in the case of more recent models, good estimates of variations in environmental chemical concentrations. Major limitations in the application of these predictive models in situations like an effluent receiving zone include virtually no information on the concentrations and duration of exposure, or the more difficult issue of estimating how long fish were present in surrounding waters where effluent exposure would not be an issue.

Limitations on the effectiveness of using tracers such as resin acids and possibly most of the chlorinated phenols examined, and the chlorinated dioxins and furans can be clarified on the basis of their kinetics. Waterborne uptake is generally viewed as the primary mode of uptake for chemical groups including the resin acids and many chlorinated phenols with properties such as high solubility and relatively short biological half-lives. Their bioconcentration factor (BCF), which is a ratio of chemical concentration in fish/water under steady-state conditions, may be used to assess the limitations of the intended use of a tracer. Estimates of BCFs available for resin acids and some chlorinated phenols suggested factors of less than 100 (Oikari et al., 1982; Niimi and Lee, 1992). Various studies have indicated the biological half-lives of most resin acids and many of the chlorinated phenols monitored to be in the order of less than a week (Niimi et al., 1990; Niimi and Lee, 1992). Chemicals with these properties should attain equilibrium concentrations in fish within a week at near steady-state exposure conditions. If near steady-state conditions occur in an effluent receiving environment, and chemical tracers such as these were detected in fish, one might conclude they were exposed for no more than a week. Under these conditions, the chemical concentrations among these fish should not differ appreciably from fish that had inhabited those waters for considerably longer periods because both are near equilibrium state. In a less than uniform environment where concentrations can vary by an order of magnitude or more, it would not be possible to predict how long fish were exposed to the effluent with any degree of confidence, particularly if no additional information is available on fish movements. Fish are efficient at accumulating waterborne chemicals, as indicated by LC_{50} studies, and this is a continuing process.

Therefore, little differences in measured concentrations may be noted in a situation where fish are exposed to a high concentration for a short period compared to a much longer exposure to a low concentration. One might speculate a variable pattern of exposure to mill effluents of different concentrations would be the norm for most nonbenthic fish species.

The use of chlorinated dioxins and furans as tracers presents other issues because of their persistence. Dioxins and furans are not natural chemicals like resin acids, but are the by-products of anthropogenic activities. Some dioxins and furans are highly toxic, and the requirement for mills that used a chlorine bleaching process to monitor these chemicals in fish was based on this concern (AEEMR, 1992b). Some mills may have been using a chlorine based process during the cycle 1 EEM program, but this practice has now ceased. Dioxins and furans will likely still be found in fish in effluent receiving areas for some time yet because of residual amounts in the sediments. Uptake of these compounds from sediment is not confirmation of effluent exposure. 2,3,7,8-Tetrachlorodibenzofuran was probably the only furan tnat might be used as a tracer because of its presence at the study site (Table 8). Its use as an effective tracer could be limited by its relatively long half-life up to 80 days in fish (Niimi, 1996). Waterborne uptake is less important than dietary uptake for persistent chemicals like this; therefore, changes in environmental concentrations would not rapidly influence chemical concentrations in tissues. Therefore, it could be difficult to assess the effectiveness of tracers with these properties in trying to establish the duration of exposure to effluents.

4.2.6. Issues Related to Tracer Measurements

The Working Group viewed the analytical issue as its most serious concern initially because it compromised the integrity of the data sets on which this assessment was based. When more than one laboratory was involved in the analyses of a tracer, intercalibration exercises could have been carried out, and quality control could have been maintained by the use of standard reference materials, "uncompromised" reference samples and "latent" duplicates. These practices were not conducted among the 3 to 5 laboratories that analyzed for each of the 5 organic chemical groups.

These concerns were not as critical for the chlorinated dioxins and furans because the method recommended, similar to ones used by reputable laboratories, was the most refined at that time. A number of other factors such as the addition of ^{13}C labelled standards to monitor sample preparation and instrument performance efficiencies, availability of certified reference materials and analyses by gas chromatography-mass spectrometry enhanced the quality of their measurements. The sampling protocol required a 50 g sample be taken while the methods protocol required only several grams for each sample; therefore, good agreement was achieved by the reported and theoretical detection limit of about 0.2 ng/kg.

The concentrations reported for resin and fatty acids, and the chlorinated phenols were viewed with less confidence. Standards such as o-methyl podocarpic acid, tricosanic acid and heneicosanoic were suggested for resin and fatty acid analyses, and fluorophenol, phenol, tribromophenol, dichloro-benzene and hexchlorobenzene were suggested for the chlorinated phenols. In practice very few laboratories were experienced with the analyses for these tracers compared to the number of laboratories experienced with analyzing for dioxins and furans (TGD, 1993). Each of these chemical groups also were analyzed in 4-5 tissues in fish and crabs (Table 4). Bile was the most common tissue monitored, and insufficient sample weight probably contributed to the large range in detection limits reported among studies. Bile is a liquid produced by the liver and stored in the gall bladder, and released into the small intestine to promote digestion. Bile is also an important medium used to excrete the metabolites of compounds such as resin acids, and cannot be considered a discrete tissue because its availability is influenced by feeding and digestive processes.

There have been efforts by some mills to address some of the issues dealing with the analytical aspects of tracer monitoring. Mill AB4 analyzed 21 individual bile samples for resin acids with most estimates below detection. Mill AB3 analyzed 45 individual bile samples in 2 fish species and detected chlorinated phenols in only 3 samples. Mill QC45 had a split sample analyzed by two laboratories. Each lab reported detection of the same 11 resin acids. Differences in concentrations reported were within 25% for 5 resin acids, within 80% for 2 acids, while the concentrations of 4 acids differed by a factor of 2-6-fold.

Concerns about analytical issues were viewed as serious by the Working Group; nevertheless, it is one of the few issues that can be addressed immediately. Sampling and sample processing protocol specifically for tracer analyses must be clearly defined (Nicholson and Fryer, 1996; Rohlf et al., 1996). Selection of tissue to be sampled should also include some weight requirements to eliminate the problem associated with obtaining sufficient samples. A substantial effort must also be made to ensure that the measurements provided by one laboratory can be accepted with the same level of confidence as those provided by other laboratories. All of the laboratories which conducted resin and fatty acid analyses on fish tissue for the EEM studies are involved with the Canadian Association of Environmental Analytical Laboratories/Standards Council of Canada, but there is not an accreditation program in place for these parameters. These issues can be resolved relatively easily because it does not include the large amount of uncertainties associated with other aspects of the tracer issue.

5.0 SUMMARY AND CONCLUSIONS

The Tracer Working Group examined the use of pulp and paper mill effluent tracers in fish during the first cycle of EEM studies. The purpose of the tracer use was to confirm the exposure of fish to the liquid phase of the effluent in the receiving environment. In total, the group received and evaluated a total of 183 data sets from 77 pulp and paper mills. The data sets included information on 100 chemicals measured in fish tissue from 27 different species of fish and crustaceans.

In summary, the Tracer Working Group findings were:

1. The measurement of chlorinated dibenzo-p-dioxins in muscle tissue of fish was not a good technique for confirming exposure of fish to pulp mill effluent.

2. The measurement of chlorinated dibenzofurans in muscle tissue of fish was not a good technique for confirming exposure of fish to pulp mill effluent. The possible exception was 2,3,7,8-TCDF. At 21 mills, this furan was present at higher concentrations in fish muscle from the study sites compared to the reference site. If a mill continues to use elemental chlorine for bleaching, this tracer might be useful but analytical costs are high.

3. The measurement of resin acids, dehydroabietic and abietic in particular, in the bile of fish was useful for confirming exposure of fish to pulp mill effluent at 9 of the 67 resin acid data sets that were reviewed by the Working Group during Cycle 1. There was considerable variability in the data and the accuracy and precision (quality) of the results reported by some mills was questionable.

4. The measurement of fatty acids in the bile, liver and liver/gall bladder of fish and in the hepatopancreas of crabs was not a useful technique for confirming exposure of fish to pulp mill effluent. Fatty acids are ubiquitous in the environment and are not specific enough the pulp mill effluents to be useful as a tracer.
5. The measurement of chlorinated phenols in the bile, liver and liver/gall bladder of fish fish and in the hepatopancreas of crabs was not a useful for confirming exposure of fish to pulp mill effluent during Cycle 1.

6. The measurement of inorganic chemicals, such as metals, phosphorus and sodium, in liver tissue of fish was not effective for confirming exposure of fish to pulp mill effluent at some mills during Cycle 1.

7. The measurement of biochemical tracers, such as ethoxyresorufin O-deethylase (EROD), in fish was used art 3 mills during Cycle 1and was not effective for confirming exposure of fish to pulp mill effluent.

8. The guidance provided for using tracers to confirm the exposure of fish to pulp and paper mill effluent for the first cycle of EEM was insufficient. As a result, the collection of fish tissue samples and measurement of effluent tracers on those tissue samples was inconsistent and led to some questionable results. This aspect of tracer use needs improvement for future EEM studies.

9. Softwoods are generally considered to be important sources of resin acids. Resin acids are also found in the effluents from mills processing primarily aspen. Secondary treatment has been shown to reduce the concentrations of resin acids in final effluents. Very little data on effluent concentrations of resin acids was reported in Cycle 1, either from mills with secondary treatment or those without.

10. A review of the literature did not identify any additional organic chemicals potentially useful as tracers.

11. Even with the use of resin acids in fish bile, there is still no technique available to address the question of the duration of the exposure of the fish to the effluent. Resin acids are accumulated, metabolized and excreted quickly by fish. A fish with resin acids in its bile may have been exposed for only a few days.

A fish without detectable resin acids may have moved out of the effluent plume a week before sampling. These limitations must be kept in mind when using these chemicals as tracers of fish exposure to pulp mill effluent.

6.0. RECOMMENDATIONS OF TRACERS FOR MILLS

6.1. GENERAL RECOMMENDATIONS

1. The tracer requirement was designed to supplement the adult fish survey, but the operational protocols were not developed at the same level of specificity as the other monitoring requirements such as the adult fish survey itself, and the benthic and toxicity surveys. Goals for tracer use must be clearly defined, possibly for a specific chemical or chemical group where required, and criteria be established to determine if the goal was met. This process should be developed and implemented before efforts to use tracers for verification are initiated again.

3. A clear understanding of the supporting role and limitations of tracers in the adult fish survey must be established. The limitations of monitoring tracers in an open environment with unrestrained organisms must be recognized in the context of the adult fish survey. Elevated concentrations of a tracer with a relative short biological half-life could suggest fish were exposed to the effluent. No defensible statement may be possible on the duration of time that the fish were in that zone because of the probability that a free swimming fish could pass through many concentration gradients. Indices such as fecundity and egg diameters would require many weeks to develop. In cases where fish are collected at a study site where tracer concentrations may be too low to serve its intended purpose, the significance of tracers is greatly reduced.

3. Based on the review of the cycle one data, the application of tracers in the adult fish survey is accepted in principle. It is recommended that site-specific decision making processes be followed to provide guidance for identifying mills where the probability of achieving the desired goal is high. Benefiting from the experiences gained in cycle 1 at the national level, site specificity must be the primary consideration.

6.2. SPECIFIC RECOMMENDATIONS

1. Resin acids appear to be the most promising tracer for softwood mills. Other potential tracers associated with softwoods must also be examined. Tracers for hardwoods and other furnish must also be identified if possible.

2. The WG recommends that the decision tree provided in Appendix 5 be followed to determine the applicability of fish tracers in subsequent cycles of EEM. The decision-making process considers such factors as whether fish at the study site can be captured in a local zone with some stability in effluent concentrations, if the mill under study uses a high percentage of softwood furnish, whether barriers separate reference and study sites and the effluent concentrations of resin acids.

3. A QA/QC program for the measurement of tracers in samples of fish tissue is required. The participation in round robin testing particularly for resin acids, chlorinated phenols, and other tracers that may be considered in the future, must be a routine practice, similar to those available for laboratories that analyze for dioxins and furans. Certified reference materials (CRMs) may not be available for many of these chemicals and, therefore, must be developed and become an integral part of the analytical validation process.

4. The use of single composite samples must be eliminated for tracer purposes to provide a statistical basis for data evaluation and decision making. Future sampling should include multiple individual samples or multiple pooled (composite) samples.

5. Use of dioxins and furans as tracers should be discontinued. It must also be recognized that current mill practices have substantially reduced, if not virtually eliminated, the discharge of dioxins and furans in

effluents. Residual amounts that may be detected in fish are likely attributable to sources deposited in sediments from previous mill practices rather than the quality of the mill effluent presently discharged.

6.3. RECOMMENDATIONS FOR FURTHER STUDIES

1. Field validation studies should be initiated prior to, or in conjunction with, the cycle 2 program, to assess the effectiveness of the revised tracer protocol. These studies should include caged fish and free-swimming fish captured nearby to compare tracer concentrations. Water samples should be taken at frequent intervals at the mill outfall and in close proximity to the fish sampling area. Intensive water sampling over a period of several days can assess daily variations in effluent exposure. Water samples must be taken during the time of fish sampling for all tracer applications. If tracer concentrations are low in water, they will likely be low in fish. If water concentrations are relatively high, and bioconcentration models suggest levels in fish should be in the measurable range, the probability of detecting measurable levels would be greater. It waterborne concentrations are relatively high at the collection site, and levels in fish are low, a conclusion can be drawn from that observation.

2. An extensive testing program must be conducted for each chemical or chemical group to determine suitable tissues to be monitored. For example, while bile was extensively used to monitor resin acids, difficulties in obtaining sufficient sample weight markedly compromised its theoretical detection limit. Tissues like muscle, liver and kidney should also be examined. The testing program should also include testing other species of aquatic organisms because of the national scope of the EEM program.

7. ACKNOWLEDGEMENTS

The Working Group would like to thank members of Environment Canada's regional staff for providing information on tracers that were included in the Cycle 1 EEM mill reports. The efforts of I. Menard to obtain and summarize this information is gratefully acknowledged. Support to the Working Group was provided by the national and regional offices of Environment Canada, Department of Fisheries and Oceans, Noranda Technology Centre, Abitibi-Price Inc. and the Canadian Pulp and Paper Association. Editing services were provided by M. Rudi and the report formatted by B. Best, both of DFO, St. Andrews Biological Station.

8.0. REFERENCES

AEEMR. 1992a. Aquatic Environmental Effects Monitoring Requirements. Environment Canada, Department of Fisheries and Oceans, May 20, 1992. EPS 1/RM/18.

AEEMR. 1992b. Aquatic Environmental Effects Monitoring Requirements. Annex 1. Aquatic environmental effects monitoring requirements at pulp and paper mills and off-site treatment facilities regulated under the pulp and paper effluent regulations of the Fisheries Act, May 20, 1992.

Chen, T., C. Breuil, S. Carriere and J.V. Hatton. 1994. Solid-phase extraction can rapidly separate lipid classes from acetone extracts of wood and pulp. Tappi J. 77: 235-240.

Cook, D.L., L. LaFleur, A. Parrish, J. Jones and D. Hoy. 1997. Characterization of plant sterols from 22 US pulp and paper mills. Wat Sci. Tech. 35: 297-303.

Ferguson, M.L., M.R. Servos, K.R. Munkittrick and J. Parrott. 1992. Inability of resin acid exposure to elevate EROD activity in rainbow trout (*Oncorhynchus mykiss*). Water Poll. Res. J. Can. 27: 561-573.

FG. 1995. Further Guidance. For the adult fish survey for aquatic environmental effects monitoring related to the Federal Fisheries Act requirements. Department of Fisheries and Oceans, Environment Canada. EEM 1, February 1995.

Healey, J., M.R. Servos and K.R. Munkittrick. 1994. Tracers of exposure of fish to pulp and paper mill effluents - a review of the published literature. Can. Tech. Rep. Fish. Aquat. Sci. No. 1929.

Koistinen, J., J. Paasivirta, T. Nevalainen and M. Lahtiperæ. 1994. Chlorphenanthrenes, alkylchlorophenanthrenes and alkychloronaphthalenes in kraft pulp mill products and discharges. Chemosphere 28: 1261-1277.

Malinowski, E.R. 1991. Factor Analysis in Chemistry. 2nd edition. John Wiley & Sons, Inc., NY.

Nicholson, M.D., and R.J. Fryer. 1996. Contaminants in marine organisms: pooling strategies for monitoring mean concentrations. ICES Techniques in Marine Environmental Sciences. No. 18, ICES, Palaegade 2-4, DK-1261, Copenhagen, Denmark.

Niimi, A.J. 1987. Biological half-lives of chemicals in fishes. Rev. Environ. Contam. Toxicol. 99: 1-46.

Niimi, A.J. 1996. Evaluation of PCBs and PCDD/Fs retention by aquatic organisms. Sci. Total Environ. 192: 123-150.

Niimi, A.J., and H.B. Lee. 1992. Free and conjugated concentrations of nine resin acids in rainbow trout (*Oncorhynchus mykiss*) following water exposure. Environ. Toxicol. Chem. 11: 1404-1407.

Niimi, A.J., H.B. Lee and G.P. Kissoon. 1990. Kinetics of chloroguaiacols and other chlorinated phenolic derivatives in rainbow trout (*Salmo gairdneri*). Environ. Toxicol. Chem. 9: 649-653.

Oikari, A.O.J. 1986. Metabolites of xenobiotics in the bile of fish in waterways polluted by pulpmill effluents. Bull. Environ. Contam. Toxicol. 36: 429-436.

Oikari, A., A. Anas, G. Kruzynski and B. Holmbom. 1984. Free and conjugated resin acids in the bile of rainbow trout, *Salmo gairdneri*. Bull. Environ. Contam. Toxicol. 33: 233-240.

Oikari, A., and B. Holmbom. 1986. Assessment of water contamination by chlorophenolics and resin acids with the aid of fish bile metabolite, pp. 252-267. Aquatic Toxicology and Environmental Fate: Ninth Volume, ASTM STP 921, T.M. Poston and R. Purdy, Eds. American Society for Testing and Materials, Philadelphia, PA.

Oikari, A., B. Holmbom, E. Anas and H. Bister. 1980. Distribution in a recipient lake and bioaccumulation in fish of resin acids from kraft pulp mill waste waters. Paperi ja Puu Special number 4a, 1980: 195-202.

Oikari, A., B. Holmbom and H. Bister. 1982. Uptake of resin acids into tissues of trout (*Salmo gairdneri* Richardson). Ann. Zool. Fennici 19: 61-64.

Plam, H., J. Paasivirta and R. Lammi. 1995. Behaviour of chlorinated phenolic compounds in bleach-plant, treatment-system and archipelago area. Chemosphere 31: 2839-2852.

Rantio, T., and J. Paasivirta. 1996. Modeled and observed fate of chlorocymenes. Chemosphere 33: 453-466.

Rohlf, F.J., H.R. Akcaya and S.P. Ferraro. 1996. Optimizing composite sampling protocols. Environ. Sci. Technol. 30: 2899-2905.

Sinkkonen, S., E. Kolehmainen, J. Paasivirta, J. Koistinen, M. Lahtiperæ and R. Lammi. 1994. Identification and level estimation of chlorinated neutral aromatic sulfur compounds and their alkylated derivatives in pulp mill effluents and sediments. Chemosphere 28: 2049-2066.

Suntio, L.R., W.Y. Shiu and D. Mackay. 1988. A review of the nature and properties of chemicals present in pulp mill effluents. Chemosphere 17: 1249-1290.

Stuthridge, T.R., K. Johnsen, H. Hoel and J. Tana. 1995. Bioaccumulation of resin acids in trout exposed to thermomechanical pulping effluents and their recipient waters. In: Proceedings of 49th APPITA Annual General Conference, Hobart, Australia.

Tavendale, M.H., I.M. Hannus, A.L. Wilkins, A.G. Langdon, K.L. Mackie and P.N. McFarlane. 1996. Bile analyses of goldfish (*Crassius auratus*) resident in a New Zealand hydrolake receiving a bleached kraft mill discharge. Chemosphere 33: 2273-2289.

TGD. 1993. Technical Guidance Document. For aquatic environmental effects monitoring related to Federal Fisheries Act requirements. Environment Canada, Department of Fisheries and Oceans, Version 1.0, April 1993.

Trapp, S., T. Rantio and J. Paasivirta. 1994. Fate of pulp mill effluent compounds in a Finnish watercourse. Environ. Sci. Pollut. Res. 1: 246-252.

Van der Weiden, M.E.J., L.P. de Vries, K. Fase, M. Celander, W. Seinen and M. Van den Berg. 1994. Relative potencies of polychlorinated dibenzo-p-dioxins (PCDD's), dibenzo-furans (PCD's) and biphenyls (PCB's), for cytochrome P450 1A induction in the mirror carp (*Cyprinus carpio*). Aquat. Toxicol. 29: 163-182

Voss, R.H. 1987. Trace organic contaminants in pulp and paper mill effluents and their environmental effects. Pulp and Paper Research Institute of Canada, Misc. Rep. MR 112, Pointe Claire, QC.

Zitko, V. 1994. Principal Component Analysis in the evaluation of environmental data. Mar. Poll. Bull. 28: 718-722.

Zender, J.A., T.R. Stuthridge, A.G. Langdon, A.L. Wilkins, K.L. Mackie and P.N. McFarlane. 1994. Removal and transformation of resin acids during secondary treatment at a New Zealand bleached kraft pulp and paper mill. Wat. Sci. Tech. 29: 105-121.

Table 1. List of the 27 species, and species code, that were monitored for tracers.

Code	Common name	Scientific name
Bur	Burbot	*Lota lota*
Car	Carp	*Cyprinus carpio*
ChC	Channel catfish	*Ictalurus punctatus*
DCr	Dungeness crab	*Cancer magister*
EnS	English sole	*Parophrys vetulus*
GCr	Green crab	*Carcinus maenas*
LaS	Largescale sucker	*Catostomus macrocheilus*
LaW	Lake whitefish	*Coregonus clupeaformis*
LhS	Longhorn sculpin	*Myoxocephalus octodecemspinosus*
LoS	Longnose sucker	*Catostomus catostomus*
Mum	Mummichog	*Fundulus heteroclitus*
MoW	Mountain whitefish	*Prosopium williamsoni*
NoP	Northern pike	*Esox lucius*
NRS	Northern redhorse sucker	*Moxostoma macrolepidotum*
PrS	Prickly sculpin	*Cottus asper*
RCr	Rock crab	*Cancer irroratus*
SmB	Smallmouth bass	*Micropterus dolomieui*
SRS	Silver redhorse sucker	*Moxostoma anisurum*
StF	Starry flounder	*Platichthys stellatus*
StS	Staghorn sculpin	*Leptocattus armatus*
SpS	Spottail shiner	*Notropis hudsonius*
Tom	Tomcod	*Microgadus tomcod*
Wal	Walleye	*Stizostedion vitreum vitreum*
WiF	Winter flounder	*Pleuronectes americanus*
WiR	Widow rockfish	*Sebastes entomelas*
WhS	White sucker	*Catostomus commersoni*
YeP	Yellow perch	*Perca flavescens*

Table 2. List of organic, inorganic and biochemicals that were monitored in biota. Organic chemicals are identified by a numeric code, inorganic chemicals by their symbol, and the biochemical by its common abbreviation.

Code	Chemicals
ORGANIC CHEMICALS	
Polychlorinated dibenzo-*p*-dioxins (DD)	
1	2,3,7,8-Tetrachloro-
2	1,2,3,7,8-Pentachloro-
3	1,2,3,4,7,8-Hexachloro-
4	1,2,3,6,7,8-Hexachloro-
5	1,2,3,7,8,9-Hexachloro-
6	1,2,3,4,6,7,8-Heptachloro-
7	Octachloro-
100	2,3-Dichloro-
101	2,7- and 2,8-Dichloro-
102	2,3,7-Trichloro-
Polychlorinated dibendofurans (DF)	
10	2,3,7,8-Tetrachloro-
11	1,2,3,7,8-Pentachloro-
12	2,3,4,7,8-Pentachloro-
13	1,2,3,4,7,8-Hexachloro-
14	1,2,3,6,7,8-Hexachloro-
15	1,2,3,7,8,9-Hexachloro-
16	2,3,4,6,7,8-Hexachloro-
17	1,2,3,4,6,7,8-Heptachloro-
18	1,2,3,4,7,8,9-Heptachloro-
19	Octachloro-
103	2,8-Dichloro-
104	2,3,8-Trichloro-
Resin acids (RA)	
20	Abietic acid
21	Dehydroabietic acid
22	Isopimaric acid
23	Levopimaric acid
24	Neoabietic acid
25	Palustric acid
26	Pimaric acid
27	Sandaracopimaric acid
28	12-Chlorodehydroabietic acid
29	14-Chlorodehydroabietic acid
30	ΣChlorodehydroabietic acid
31	12,14-Dichlorodehydroabietic acid
Fatty acids	
40	Oleic acid
41	Linolenic acid
42	Arachidic acid
43	Behenic acid
44	Lauric acid
45	Lignoceric acid
46	Linoleic acid
47	Myristic acid
48	Palmitic acid
49	Stearic acid
50	Dichlorooctadecanoic acid
51	9,10-Dichlorostearic acid
52	Palmitoleic acid
Chlorinated Phenols (CP), Subgroup: Chlorophenols	
60	2-Chlorophenol
61	4-Chlorophenol
62	2,4-Dichlorophenol
63	2,6-Dichlorophenol
64	2,4,5-Trichlorophenol
65	2,4,6-Trichlorophenol
66	2,3,4,6-Tetrachlorophenol
67	Pentachlorophenol
108	2,3-Dichlorophenol
109	3,4-Dichlorophenol
110	3,5-Dichlorophenol
111	2,3,4-Trichlorophenol
112	2,3,5-Trichlorophenol
113	2,3,6-Trichlorophenol
114	3,4,5-Trichlorophenol
115	2,3,4,5-Tetrachlorophenol
Subgroup: Chloroguaiacols	
105	Guaiacol
70	4-Chloroguaiacol
106	5-Chloroguaiacol
107	6-Chloroguaiacol
71	3,4-Dichloroguaiacol
72	4,5-Dichloroguaiacol
73	4,6-Dichloroguaiacol
74	3,4,5-Trichloroguaiacol
75	3,4,6-Trichloroguaiacol
76	4,5,6-Trichloroguaiacol
77	Tetrachloroguaiacol
Subgroup: Chlorocatecols	
78	4-Chlorocatechol
79	3,4-Dichlorocatechol
80	3,5-Dichlorocatechol
81	3,6-Dichlorocatechol
82	4,5-Dichlorocatechol
83	3,4,5-Trichlorocatechol
84	3,4,6-Trichlorocatechol
85	Tetrachlorocatechol
Subgroup: Chloroveratroles	
86	4,5-Dichloroveratrole
87	3,4,5-Trichloroveratrole
88	Tetrachloroveratrole
Subgroup: Other chlorinated phenols	
90	5-Chlorovanillin
91	6-Chlorovanillin
92	5,6-Dichlorovanillin
93	2-Chlorosyringaldehyde
94	2,6-Dichlorosyringaldehyde
95	4,5,6-Trichlorosyringol
96	Trichlorotrimethoxybenzene
INORGANIC CHEMICALS	
Cu	Copper
Fe	Iron
Mn	Manganese
Hg	Hercury
P	Phosphorus
Sr	Strontium
Zn	Zinc
BIOCHEMICALS	
EROD	Ethxyresorufin *O*-deethylase

Table 3. Number of mills that used each species for monitoring organic chemicals as tracers, and the frequency of each species used for each chemical group.

Common name	No. of mill reports	Chemical group				
		Dibenzo-p-dioxins	Dibenzo-furans	Resin acids	Fatty acids	Chlorinated phenols
White sucker	29	10	10	19	12	3
Longnose sucker	8	2	2	7		2
Winter flounder	7		7	6		
Walleye	8	8	8	1		
Tomcod	4	2	2	5	5	
Yellow perch	4	3	3	2		1
Northern RH sucker	3	1	1	3	1	
Green crab	3			3	3	
Mountain whitefish	3	2	2	3	3	1
Longhorn sculpin	2			2	2	
Rock crab	2	1	1	1	2	
Staghorn sculpin	2			2	1	
Burbot	1					1
Carp	1			1	1	
Channel catfish	1	1	1			1
Dungeness crab	1					1
English sole	1					1
Largescale sucker	1	1	1	1		1
Lake whitefish	1			1		1
Mummichog	1			1	1	
Northern pike	1	1	1			
Prickly sculpin	1			1	1	
Smallmouth bass	1	1	1			
Silver RH sucker	1			1	1	
Starry flounder	1			1	1	
Spottail shiner	1			1	1	
Widow rockfish	1	1	1			

Table 4. Tissues that were monitored for different organic chemical groups in the EEM program.

Chemical group	No. of data sets	Tissues monitored				
		Muscle	Liver	Bile	Liver/ gallbladder	Hepatopancreas
Dioxins	34	34				
Furans	34	34				
Resin acids	63	1	13	39	6	4
Fatty acids	38		6	21	6	5
Cl phenols	15	2	8	4		1

Table 5. Laboratories that measured various organic chemicals in EEM studies. Laboratories used were Canviron (C), Enviro-Test (E), Fenwick (F), Novamann (N), Wellington (W) and Zenon (Z).

Chemical group	No. of data sets	Laboratory					
		C	E	F	N	W	Z
Dioxins	29	1	6	2		8	12
Furans	29	1	6	2		8	12
Resin acids	49		16	5	16		12
Fatty acids	29		1	5	14		9
Cl phenols	8		5		2		1

Table 6. Dioxins that were used as tracers in muscle tissue of fish and crabs. Seven dioxin congeners were measured at each site. The information presented lists the mills studies; species examined; number of congeners detected at the reference and study sites for each data set; number of congener concentrations that were greater at reference than study sites, or greater at study than reference sites; the same comparison based on a 2- and 5-fold difference between sites; and number of same congeners detected at both sites.

		Detected		>		2X		5 X		
Mill	Spec.	R	S	R	S	R	S	R	S	Same
NB10	WhS	2	2	2	2	0	2			2
QC24	WhS	0	0							
ON3	WhS	0	0							
ON4	WhS	2	2	0	2					2
ON6	WhS	0	2	0	2					0
ON8	WhS	0	0							
ON15	WhS	0	0							
ON20	WhS	0	1	0	1					0
ON23	WhS	1	0	1	0	1	0	1	0	0
SK1	WhS	0	0							
QC23	Wal	2	3	2	1					2
QC27	Wal	0	0							
QC49	Wal	3	3	1	2					3
QC50	Wal	0	0							
ON10	Wal	2	3	0	3	0	3			2
ON20	Wal	0	0							
SK1	Wal	0	0							
AB3	Wal	0	0							
ON12	LoS	2	2	1	0	1	0			2
AB7	LoS	0	1	0	1					0
NB3	YeP	0	0							
QC37	YeP	2	1	2	0	1	0			1
ON8	YeP	0	0							
NB5	Tom	0	0							
NB9	Tom	1	0	1	0					0
ON9	NRS	0	2	0	2	0	1			0
QC8	NoP	3	3	1	2	0	1			3
QC24	SmB	0	0							
QC31	ChC	3	3	1	2	0	1			3
AB6	MoW	1	2	0	2	0	1			1
AB7	MoW	1	2	1	1	1	1	1	1	1
BC1	WiR	2	1	2	0	2	0			1
BC9	LaS	0	1	0	1					0
NS6	RCr	0	0							

Table 7. Concentrations of dioxins, in ng/kg, in muscle tissue of fish. *DD = dibenzodioxin (see Table 2).

		Reference site				Study site			
Mill	Spec.	1*	2	6	7	1	2	6	7
NB10	WhS			0.46	0.58			0.48	0.66
ON4	WhS			0.77	23.00			1.10	35.00
ON6	WhS							0.20	0.40
ON20	WhS							0.30	
ON23	WhS			5.30					
QC23	Wal			1.10	4.00		0.37	0.76	3.10
QC49	Wal		0.40	0.60	0.80		0.40	0.50	0.90
ON10	Wal			0.61	2.10	0.66		0.79	3.90
ON12	LoS			0.79	19.00			0.79	7.80
AB7	LoS					0.30			
QC37	YeP		0.60		2.30				1.40
NB9	Tom	0.20							
ON9	NRS					0.30		0.60	
QC8	NoP		0.32	0.42	0.62		0.29	0.54	2.50
QC31	ChC		0.40	0.60	0.93		0.40	0.60	1.50
AB6	MoW				1.00	0.80			1.30
AB7	MoW				1.00	1.20			0.70
BC1	WiR			1.70	4.50				4.20
BC9	LaS					0.20			

Table 8. Furans that were used as tracers in muscle tissue of fish and crabs. Nine furan congeners were measured at each site. The information presented lists the mills studies; species examined; number of congeners detected at the reference and study sites for each data set; number of congener concentrations that were greater at reference than study sites, or greater at study than reference sites; the same comparison based on a 2- and 5-fold difference between sites; and number of same congeners detected at both sites.

		Detected		>		2X		5X		10X		
Mill	Spec.	R	S	R	S	R	S	R	S	R	S	Same
NB10	WhS	0	1	0	1	0	1					0
QC24	WhS	0	1	0	1	0	1					0
ON3	WhS	0	1	0	1	0	1	0	1	0	1	0
ON4	WhS	1	0	1	0	1	0					0
ON6	WhS	2	1	2	0							1
ON8	WhS	1	5	0	5	0	5	0	2			1
ON15	WhS	1	1	0	1	0	1					1
ON20	WhS	1	1	0	1							1
ON23	WhS	2	1	2	1							0
SK1	WhS	0	0									
QC23	Wal	0	0									
QC27	Wal	0	0									
QC49	Wal	1	1	0	1							1
QC50	Wal	0	0									
ON10	Wal	1	2	0	2	0	2	0	2			1
ON20	Wal	0	1	0	1							0
SK1	Wal	0	0									
AB3	Wal	0	0									
ON12	LoS	2	2	1	1							2
AB7	LoS	1	1	0	1	0	1	0	1	0	1	1
NB3	YeP	0	0									
QC37	YeP	3	4	1	2	1	1					3
ON8	YeP	2	2	1	2	1	2					1
NB5	Tom	0	0									
NB9	Tom	1	0	1	0	1	0					0
ON9	NRS	0	1	0	1	0	1					0
QC8	NoP	2	3	0	2							2
QC24	SmB	0	1	0	1	0	1					0
QC31	ChC	2	3	0	3	0	1					2
AB6	MoW	1	1	0	1	0	1					1
AB7	MoW	1	1	0	1	0	1					1
BC1	WiR	5	1	4	1	4	0					1
BC9	LaS	0	1	0	1	0	1	0	1	0	1	0
NS6	RCr	0	0									

Table 9. Concentrations of furans, in ng/kg, in muscle tissue of fish. *DF = dibenzofuran (see Table 2).

		Reference site								Study site								
Mill	Spec.	10*	11	12	13	14	15	17	19	10	11	12	13	14	15	16	17	19
NB10	WhS									0.50								
QC24	WhS									1.00								
ON3	WhS									5.00								
ON4	WhS			0.88														
ON6	WhS	0.80		0.20						0.50								
ON8	WhS	0.80								2.40	0.5			0.90	1.60	1.70		
ON15	WhS	0.65								1.80								
ON20	WhS	0.30								0.40								
ON23	WhS			0.30			0.40			0.20								
QC49	Wal								0.21									0.31
ON10	Wal	0.37								2.30		1.50						
ON20	Wal									0.20								
ON12	LoS	2.10		4.90						4.00		4.20						
AB7	LoS	0.10								3.80								
QC37	YeP	0.40					0.60	0.30		0.80					0.20		0.30	0.30
ON8	YeP	0.20					0.80			0.50						0.40		
NB9	Tom	0.50																
ON9	NRS									1.00								
QC8	NoP				0.20				0.20	0.20			0.20					0.27
QC24	SmB									1.00								
QC31	ChC		0.20						0.40		0.30			0.39				0.80
AB6	MoW	1.40								3.30								
AB7	MoW	1.40								6.70								
BC1	WiR	1.90			2.70	1.20		4.60	3.10	2.70								
BC9	LaS									3.30								

Table 10. Resin acids that were used as tracers in muscle tissue of fish and crabs. Nine resin acids were measured at each site. The information presented lists the mills studies; species examined; number of congeners detected at the reference and study sites for each data set; number of congener concentrations that were greater at reference than study sites, or greater at study than reference sites; the same comparison based on a 2- and 5-fold difference between sites; and number of same congeners detected at both sites.

				Detected		>		2X		5X		10X		100X		
Mill	RAs	Spec.	Tiss.	R	S	R	S	R	S	R	S	R	S	R	S	Same
NB3	11	WhS	B	5	7	1	7	0	6	0	4	0	2			4
NB10	8	WhS	B	1	1	1	0									1
QC7	11	WhS	B	1	0	1	0									0
QC11	10	WhS	B	1	0	1	0									0
QC12	10	WhS	B	2	5	0	5	0	4	0	2					2
QC13	10	WhS	B	0	1	0	1	0	1							0
QC15	10	WhS	B	0	5	0	5	0	4	0	2	0	1			0
QC16	10	WhS	B	1	1	1	0									1
QC17	10	WhS	B	2	1	1	0	1	0							1
QC34	10	WhS	B	0	1	1	0	1	0	1	0					0
QC45	11	WhS	B	2	4	1	3	0	2							2
ON21	8	WhS	B	1	2	0	2	0	2	0	2	0	2	0	2	1
ON22	8	WhS	B	0	1	0	1	0	1	0	1					0
AB5	11	WhS	B	1	1	0	1	0	1	0	1					1
QC46	10	WhS	L	1	1	0	0									1
QC48	10	WhS	L	0	1	0	1	0	1	0	1	0	1			0
ON16	8	WhS	L	0	0											
ON18	8	WhS	L	0	0											
SK1	5	WhS	M	0	0											
NF2	8	WiF	B	0	0											
NF3	8	WiF	B	1	1	0	1	0	1							1
NS1	8	WiF	B	1	0											0
NB11	8	WiF	B	1	1	1	0	1	0							1
NS4	8	WiF	L	0	0											
NS6	5	WiF	L	0	0											
QC30	10	WiF	L	0	0											
QC14	11	LoS	B	1	4	0	4	0	4	0	3	0	2			1
QC23	10	LoS	B	1	9	0	9	0	9	0	7	0	7	0	2	1
AB3	11	LoS	B	0	0											
AB4	11	LoS	B	3	3	0	2	0	2	0	1					1
AB6	11	LoS	B	0	3	0	3	0	3							0
AB7	11	LoS	B	0	2	0	2									0
BC8	11	LoS	B	0	1	0	1									0
QC16	10	NRS	B	1	1	0	1									1
QC35	10	NRS	B	0	1	0	1	0	1							0
ON9	11	NRS	B	1	5	0	5	0	5	0	5	0	5			1
QC15	10	SRS	B	1	6	0	6	0	6	0	2	0	2			1
NS5	8	Tom	L/GB	0	0											
NB9	11	Tom	L/GB	9	8	4	4	1	2	0	2	0	1			8
NB4	11	Tom	L/GB	3	5	3	4	3	3	3	2	2	1	1	0	1

Table 10. (cont'd)

				Detected		>		2X		5X		10X		100X		Same
Mill	RAs	Spec.	Tiss.	R	S	R	S	R	S	R	S	R	S	R	S	
NB8	11	Tom	L/GB	5	6	2	4	1	4	1	2	1	1			5
NB5	11	Tom	L/GB	2	1	2	1	1	1	1	1	1	1			0
AB6	11	MoW	B	7	2	7	0	7	0	5	0	5	0			2
AB7	11	MoW	B	7	9	7	3	7	0	5	0	5	0			6
BC8	11	MoW	B	3	4	1	2	0	1							3
NB4	11	GCr	H	0	4	0	4	0	3	0	1	0	1			0
NB5	11	GCr	H	2	3	1	3	1	3	1	3	1	3			1
NB7	11	GCr	H	5	7	2	6	1	6	0	5	0	5	0	2	4
NS1	8	LhS	B	1	0	1	0	1	0	1	0					0
NS2	8	LhS	B	2	4	0	4	0	4	0	3	0	2			2
NB3	11	YeP	B	2	8	0	8	0	7	0	5	0	5			2
QC36	11	YeP	L	1	4	0	4	0	4	0	4	0	4			1
BC6	10	StS	L	7	0	7	0									0
BC7	10	StS	B	2	2	0	1									2
BC7	10	PrS	B	2	2	2	0									2
BC7	10	StF	B	0	1	0	1									0
BC9	5	LaW	L	1	0	1	0	1	0	1	0	1	0			0
NB5	11	Mum	L/GB	3	3	2	3	2	3	1	2	1	2			1
QC50	11	Wal	B	1	7	0	7	0	6	0	6	0	4	0	2	1
ON18	8	SpS	L	1	0	1	0	1	0	1	0	1	0			0
BC9	5	LaS	L	0	0											
NS6	5	RCr	H	0	0											
ON5	8	Car	L	0	0											

Table 11. Concentrations of resin acids, in µg/g, in tissues of fish. *RA = resin acid (see Table 2).

			Reference site											Study site											
			20*	21	22	23	24	25	26	27	28	29	31	20	21	22	23	24	25	26	27	28	29	30	31
NB3	WhS	B		0.24	0.05					0.26	2.6	0.2		3	8.9					1.8	1.5	32	3.5		0.25
NB10	WhS	B		1											0.75										
QC7	WhS	B		0.39																					
QC11	WhS	B		0.55																					
QC12	WhS	B	0.49	0.69										3	6.5	1.4		1.9	0.47						
QC13	WhS	B													1.2				0.28						
QC15	WhS	B												3.8	11	1.1		1.1							
QC34	WhS	B												3.1											
QC16	WhS	B		2											1.8										
!C17	WhS	B		0.93			1.5								1.2										
QC45	WhS	B	1.5	10.8										3.7	4.9	0.63			0.96						
QC46	WhS	L		0.5											0.5										
QC48	WhS	L													13										
ON21	WhS	B		0.4										71	110										
ON22	WhS	B													2.6										
AB5	WhS	B		0.17											0.34										
NF3	WiF	B		0.1											0.3										
NS1	WiF	B		3.3																					
NB11	WiF	L		0.8											0.2										
QC14	LoS	B		0.17											33	3.3				8.6	1.7				
QC23	LoS	B							25					30	63	23		9.6	9	86	14			11	1.2
AB4	LoS	B			0.1		0.1	0.1							0.24	0.6				0.1					
AB6	LoS	B													0.16	0.12				0.52					
AB7	LoS	B												0.45						0.44					
BC8	LoS	B													0.15										
QC16	NRS	B		1.6											2.4										
QC35	NRS	B												2.7											
ON9	NRS	B		0.26										13	3.7	4.9		19			4.5				
QC15	SRS	B					1							2.2	5.8	5.4	1.2	1.3	1						
NB4	Tom	L/GB	4		16				176								3.6	0.23	1.5	0.6	9.5				
NB5	Tom	L/GB	11				0.46													41					
NB6	Tom	L/GB	28			0.48	0.2		0.7	0.4				1.8			29	0.27	1.3	5.4	0.09				
NB9	Tom	L/GB	29	65	16		1300	2.6	1380	220	11	3.7		9	610	312		124	3.4	130	221	11			

Table 11. (cont'd)

			Reference site											Study site											
			20*	21	22	23	24	25	26	27	28	29	31	20	21	22	23	24	25	26	27	28	29	30	31
AB7	MoW	B	20	27	13	6.6	1.1	15		1.4				0.26	0.7	0.35		0.06	0.11	0.37	0.09	0.07	0.18		
BC8	MoW	B	0.18	0.46	0.3									0.72	0.3	0.36					0.15				
NB4	Gcr	H												0.77			18		0.16	0.68					
NB5	Gcr	H			1.7		12							7.1		22			10						
NB7	Gcr	H	86		0.6	199	0.4		0.2					31	23	77	544		44	413	16				
NS1	LhS	B		3.9																					
NS2	LhS	B		3.5	2									7.6	16	16	6.9								
NB3	YeP	B		0.51						3.2				12	23	13	1.3		8.6		5	10	0.95		
QC36	YeP	L		0.51											9.4	35	8				14				
BC6	StS	L	0.12	0.35	0.26	0.1					0.24	0.26	0.3												
BC7	StS	B		1.1				0.59							1.3				0.59						
BC7	PrS	B		1.3				0.64							1.2				0.56						
BC7	StF	B																	0.74						
BC9	LaW	L		10																					
NB5	Mum	L/GB	1.7		16			2.4									11		11		10				
QC50	Wal	B		0.2											280	8.1				11	3.5	62	14		0.3
ON18	SpS	L		12																					

Table 12. Resin acid in fish tissue - ratio of study site value to reference site value. *RA = resin acid (see Table 2).

Mill	Spec.	Tiss.	S/R 20*	S/R 21	S/R 22	S/R 23	S/R 24	S/R 25	S/R 26	S/R 27	S/R 28	S/R 29	S/R 31
AB4	LoS	B	1.0	2.4	6.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
AB5	WhS	B	1.0	2.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
AB6	LoS	B	1.0	1.6	1.2	1.0	1.0	1.0	5.2	1.0	1.0	1.0	1.0
AB6	MoW	B	0.0	0.0	0.0	0.0	0.1	0.0	1.0	0.1	1.0	1.0	1.0
AB7	LoS	B	4.5	1.0	1.0	1.0	1.0	1.0	4.4	1.0	1.0	1.0	1.0
AB7	MoW	B	0.0	0.0	0.0	0.0	0.1	0.0	3.7	0.1	0.7	1.8	1.0
BC6	StS	L	0.8	0.3	0.4	1.0	1.0	1.0	1.0	1.0	0.4	0.4	0.4
BC7	StS	B	1.0	1.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
BC7	PrS	B	1.0	0.9	1.0	1.0	1.0	0.9	1.0	1.0	1.0	1.0	1.0
BC7	StF	B	1.0	1.0	1.0	1.0	1.0	7.4	1.0	1.0	1.0	1.0	1.0
BC8	LoS	B	1.0	1.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
BC8	MoW	B	4.0	0.7	1.2	1.0	1.0	1.0	1.0	1.5	1.0	1.0	1.0
BC9	LaW	L	1.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NB10	WhS	B	1.0	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NB11	WiF	L	1.0	0.3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NB3	WhS	B	30.0	37.1	2.0	1.0	1.0	1.0	18.0	5.8	12.3	17.5	2.5
NB3	YeP	B	120.0	45.1	130.0	13.0	1.0	86.0	1.0	1.6	100.0	9.5	1.0
NB4	Tom	L/GB	0.0	1.0	0.0	36.0	2.3	15.0	0.0	95.0	1.0	1.0	1.0
NB4	GCr	H	7.7	1.0	1.0	180.0	1.0	1.6	6.8	1.0	1.0	1.0	1.0
NB5	Tom	L/GB	0.0	1.0	1.0	1.0	0.2	1.0	410.0	1.0	1.0	1.0	1.0
NB5	GCr	H	71.0	1.0	12.9	1.0	0.0	100.0	1.0	1.0	1.0	1.0	1.0
NB5	Mum	L/GB	0.1	1.0	0.0	110.0	1.0	4.6	1.0	100.0	1.0	1.0	1.0
NB6	Tom	L/GB	0.1	1.0	1.0	60.4	1.4	13.0	7.7	0.2	1.0	1.0	1.0
NB7	GCr	H	0.4	230.0	128.3	2.7	0.3	440.0	2065.0	160.0	1.0	1.0	1.0
NB9	Tom	L/GB	0.3	9.4	19.5	1.0	1.0	1.3	0.9	1.0	1.0	0.0	1.0
NF3	WiF	B	1.0	3.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NS1	WiF	B	1.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NS1	LhS	B	1.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NS2	LhS	B	76.0	4.6	8.0	69.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
ON18	SpS	L	1.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
ON21	WhS	B	710.0	275.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
ON22	WhS	B	1.0	26.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
ON9	NRS	B	130.0	14.2	49.0	1.0	190.0	1.0	1.0	45.0	1.0	1.0	1.0
QC11	WhS	B	1.0	0.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
QC12	WhS	B	6.1	9.4	14.0	1.0	19.0	4.7	1.0	1.0	1.0	1.0	1.0
QC13	WhS	B	1.0	12.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
QC14	LoS	B	1.0	194.1	33.0	1.0	1.0	1.0	86.0	17.0	1.0	1.0	1.0
QC15	WhS	B	38.0	110.0	11.0	1.0	11.0	2.8	1.0	1.0	1.0	1.0	1.0
QC15	SRS	B	22.0	58.0	54.0	12.0	1.3	10.0	1.0	1.0	1.0	1.0	1.0
QC16	WhS	B	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
QC16	NRS	B	1.0	1.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
QC17	WhS	B	1.0	1.3	1.0	1.0	0.1	1.0	1.0	1.0	1.0	1.0	1.0
QC23	LoS	B	300.0	630.0	230.0	1.0	96.0	90.0	3.4	140.0	1.0	1.0	12.0
QC34	WhS	B	31.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
QC35	NRS	B	27.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
QC36	YeP	L	1.0	18.4	350.0	80.0	1.0	1.0	1.0	140.0	1.0	1.0	1.0
QC45	WhS	B	2.5	0.5	6.3	1.0	1.0	9.6	1.0	1.0	1.0	1.0	1.0
QC46	WhS	L	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
QC48	WhS	L	1.0	130.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
QC50	Wal	B	1.0	1400.0	81.0	1.0	1.0	1.0	110.0	35.0	620.0	140.0	3.0
QC7	WhS	B	1.0	0.3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Table 13. Fatty acids that were used as tracers in fish and crab tissue. The information reported identifies the mill studies, number of fatty acids monitored, species examined, tissue sampled, number of fatty acids detected at the reference and study sites for each data set, number of fatty acids concentrations that were greater at reference than study sites, or greater at study than reference sites, that same comparisons based on a 2, 5, 10 and 100 fold difference between sites, and number of the fatty acids detected at both sites.

				Detected		>		2X		5X		10X		100X		
Mill	FAs	Spec.	Tiss.	R	S	R	S	R	S	R	S	R	S	R	S	Same
NB10	2	WhS	B	1	1	1	0									1
QC11	11	WhS	B	8	8	8	0	4	0	2	0	1	0			8
QC12	11	WhS	B	10	9	2	7	0	5	0	2	0	1			9
OC13	11	WhS	B	8	8	4	3	0	1							8
QC15	11	WhS	B	9	9	1	8	0	7	0	3	0	1			9
QC17	11	WhS	B	10	10	2	7	0	4	0	1					10
QC34	10	WhS	B	8	8	7	1	7	0							8
QC45	10	WhS	B	9	9	8	1	8	0	4	0	2	0			9
ON21	2	WhS	B	2	1	2	0	2	0	1	0	1	0	1	0	1
ON22	2	WhS	B	1	1	1	0									1
ON16	2	WhS	L	1	1	1	0									1
ON18	2	WhS	L	1	1	0	1									1
NF2	4	WiF	B	2	2	0	2	0	1							2
NF3	2	WiF	B	1	1	1	0									1
NS1	2	WiF	B	1	1	0	1									1
NS4	2	WiF	B	1	1	0	1									1
NS6	2	WiF	L	0	0											
NB11	2	WiF	L	1	1	0	1									1
NS5	2	Tom	L/GB	1	1	0	1									1
NB4	9	Tom	L/GB	7	6	6	1	5	0	4	0	4	0	1	0	6
NB5	9	Tom	L,GB	9	6	9	0	9	0	3	0	2	0			6
NB8	9	Tom	L/GB	8	8	3	5	3	2							8
NB9	9	Tom	L/GB	6	5	4	2	1	0	1	0	1	0			5
NS1	2	LhS	B	1	1	1	0									1
NS2	2	LhS	B	1	1	0	1									1
NB4	9	GCr	H	9	9	7	2	6	1	1	1	1	1			9
NB5	9	GCr	H	9	9	3	6	2	2	1	1	1	1			9
NB7	9	GCr	H	8	8	5	3	4	1							8
NS4	2	RCr	H	1	1	1	0									1
NS6	2	RCr	H	0	0											
QC15	11	SRS	B	8	8	6	2	5	0	2	0					8
QC35	11	NRS	B	8	8	5	3									8
BC7	11	StS	B	3	3	0	3	0	3	0	1					3
BC7	11	PrS	B	3	3	0	3	0	2							3
BC7	11	StF	B	3	3	1	2									3
NB5	9	Mum	L/GB	9	8	9	0	6	0	3	0	1	0			8
ON5	2	Car	L	1	1	0	1									1
ON18	2	SpS	L	1	1	0	1									1

Table 14. Concentrations of fatty acids, in mg/kg. *FA = fatty acid (see Table 2).

			Reference site										Study site									
Mill	Spec.	Tiss.	40*	41	42	43	44	46	47	48	49	50	40	41	42	43	44	46	47	48	49	50
NB10	WhS	B	179										154									
QC11	WhS	B	380	95	30		250	140	110	470	170		340	49	15		15	120	18	270	110	
QC12	WhS	B	300	45	12	1.3	5.4	210	35	390	200		1700	88	12	0.7	63	660	180	840	260	
QC13	WhS	B	4130	170	63		40	770	188	1240	420		2760	150	63		48	900	180	2760	380	
QC15	WhS	B	760	90	9.2	0.9	0.89	100	51	600	100		2200	69	23	1.3	25	540	280	1900	420	
QC17	WhS	B	490	100	11	0.8	63	490	120	1600	120		2100	100	56	2.2	33	530	250	1300	340	
QC34	WhS	B	3030	230	52		137	1230	670	2370	770		1370	103	57		31	610	139	880	330	
QC45	WhS	B	477	110	23	1.3	35	177	70	587	180		80	14	5.8	1.6	0.7	41	6.6	179	74	
ON21	WhS	B	449	294									233									
ON22	WhS	B	88										54									
ON16	WhS	L	8900										8500									
ON18	WhS	L	1700										7200									
NF2	WiF	B	120							80			13							172		
NF3	WiF	B	170										110									
NS1	WiF	B	94										75									
NS4	WiF	B	2940										3860									
NB11	WiF	L	424										733									
NS5	Tom	L/GB	3120										4950									
NB4	Tom	L/GB	448	867		2.9	0.76		475	1660	856		22	23			4.5			244	134	7.4
NB5	Tom	L/GB	556	509	4.2	5.3	3.9		862	3960	1370	46	278	230			1.9		295	1780	592	
NB8	Tom	L/GB	101	234	1.3		11		22	494	220	3.4	69	68	5.1		28		90	490	386	11
NB9	Tom	L/GB	1360	1550					43	1200	945	9.1	815	1525						1140	980	9.6
NS1	LhS	B	94										75									
NS2	LhS	B	40										69									
NB4	GCr	H	331	242	46	4.2	1.8		791	2493	1330	51	650	145	16	0.1	60		281	896	438	11
NB5	GCr	H	227	58	242	19	1.3		490	1660	782	38	690	664	12	21	1.6		573	1980	630	14
NB7	GCr	H	798	693	889	45			1130	1820	935	54	340	214	2030	86			314	2980	211	82
NS4	Rcr	H	6500										5430									

Table 14. (cont'd)

			Reference site										Study site									
Mill	Spec.	Tiss.	40*	41	42	43	44	46	47	48	49	50	40	41	42	43	44	46	47	48	49	50
QC15	SRS	B	410	52	6.5		20	410	29	560	320		570	6.1	6		7.8	83	54	70	110	
QC35	NRS	B	1200	160	22		100	140	150	860	250		800	270	30		90	150	100	620	190	
BC7	StS	B		45				67	31					130				210	210			
BC7	PrS	B		86				190	260					170				390	680			
BC7	StF	B		21				52	65					30				60	46			
NB5	Mum	L/GB	1330	855	33	1.9	28		1110	2430	1000	35	336	354	1.5		5		758	1690	574	1.6
ON5	Car	L	5600										8500									
ON18	SpS	L	1100										233									

Table 15. Fatty acids in fish tissues - ratio of study site concentration to reference site concentration. *FA = fatty acid (see Table 2).

Mill	Spec.	Tiss.	S/R 40*	S/R 41	S/R 42	S/R 43	S/R 44	S/R 46	S/R 47	S/R 48	S/R 49	S/R 50
NB10	WhS	B	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
QC11	WhS	B	0.9	0.5	0.5	1.0	0.1	0.9	0.2	0.6	0.6	1.0
QC12	WhS	B	5.7	2.0	1.0	0.5	11.7	3.1	5.1	2.2	1.3	1.0
QC13	WhS	B	0.7	0.9	1.0	1.0	1.2	1.2	1.0	2.2	0.9	1.0
QC15	WhS	B	2.9	0.8	2.5	1.5	28.1	5.4	5.5	3.2	4.2	1.0
QC17	WhS	B	4.3	1.0	5.1	2.8	0.5	1.1	2.1	0.8	2.8	1.0
QC34	WhS	B	0.5	0.4	1.1	0.0	0.2	0.5	0.2	0.4	0.4	1.0
QC45	WhS	B	0.2	0.1	0.3	1.2	0.0	0.2	0.1	0.3	0.4	1.0
ON21	WhS	B	0.5	0.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
ON22	WhS	B	0.6	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
ON16	WhS	L	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
ON18	WhS	L	4.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NF2	WiF	B	0.1	1.0	1.0	1.0	1.0	1.0	1.0	2.2	1.0	1.0
NF3	WiF	B	0.6	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NS1	WiF	B	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NS4	WiF	B	1.3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NB11	WiF	L	1.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NS5	Tom	L/GB	1.6	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NB4	Tom	L/GB	0.0	0.0	1.0	0.0	5.9	1.0	0.0	0.1	0.2	74.0
NB5	Tom	L/GB	0.5	0.5	0.0	0.0	0.5	1.0	0.3	0.4	0.4	0.0
NB8	Tom	L/GB	0.7	0.3	3.9	1.0	2.5	1.0	4.1	1.0	1.8	3.2
NB9	Tom	L/GB	0.6	1.0	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.1
NS1	LhS	B	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NS2	LhS	B	1.7	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NB4	GCr	H	2.0	0.6	0.3	0.0	33.3	1.0	0.4	0.4	0.3	0.2
NB5	GCr	H	3.0	11.4	0.0	1.1	1.2	1.0	1.2	1.2	0.8	0.4
NB7	GCr	H	0.4	0.3	2.3	1.9	1.0	1.0	0.3	1.6	0.2	1.5
NS4	RCr	H	0.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
QC15	SRS	B	1.4	0.1	0.9	1.0	0.4	0.2	1.9	0.1	0.3	1.0
QC35	NRS	B	0.7	1.7	1.4	1.0	0.9	1.1	0.7	0.7	0.8	1.0
BC7	StS	B	1.0	2.9	1.0	1.0	1.0	3.1	6.8	1.0	1.0	1.0
BC7	PrS	B	1.0	2.0	1.0	1.0	1.0	2.1	2.6	1.0	1.0	1.0
BC7	StF	B	1.0	1.4	1.0	1.0	1.0	1.2	0.7	1.0	1.0	1.0
NB5	Mum	L/GB	0.3	0.4	0.0	0.1	0.2	1.0	0.7	0.7	0.6	0.0
ON5	Car	L	1.5	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
ON18	SpS	L	0.2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Table 16. Chlorinated phenols in fish tissue. The information reported identifies the mill studies, number of chlorinated phenols monitored, species examined, tissue sampled, number of chlorinated phenold detected at the reference and study sites for each data set, number of chlorinated phenol concentrations that were greater at reference than study sites, or greater at study than reference sites, that same comparisons based on a 2, 5, 10 and 100 fold difference between sites, and number of the same chlorinated phenols detected at both sites.

				Detected		>		2X		5X		10X		100X		
Mill	Cps	Spec.	Tiss.	R	S	R	S	R	S	R	S	R	S	R	S	Same
QC31	10	WhS	L	3	4	0	4	0	1							3
QC49	10	WhS					Reported same data as QC31									
ON16	10	WhS	L	3	3	2	1	2	0	2	0	2	0	1	0	1
SK1	23	WhS	M	0	1	0	1	0	1							0
AB3	34	LoS	B	0	1	0	1									0
AB6	34	LoS	B	2	4	1	4	0	1							1
QC31	10	ChC	L	3	1	3	0	3	0							1
QC37	34	YeP	L	0	2	0	2	0	2	0	2	0	2			0
SK1	23	Wal	M	0	0											
AB3	34	Bur	B	0	1	0	1	0	1							0
AB6	34	MoW	B	0	2	0	2									0
BC9	33	LaW	L	0	0											
BC9	33	LaS	L	0	0											
BC13	36	EnS	L	0	0											
BC13	36	DCr	H	2	1	2	0	2	0	1	0					1

Table 17. Concentration of chlorinated phenols, in µg/g. *CP = chlorinated phenol (see Table 2).

			Reference site								Study site										
Mill	Spec.	Tiss.	70*	72	74	76	77	83	105	107	64	70	71	72	74	76	77	83	88	96	105	107
QC31	WhS	L		35	70		5							55	80	10	8					
ON16	WhS	L	13500						160	2040		80									160	140
SK1	WhS	L																	3			
AB3	LoS	B																58				
AB6	LoS	B				12		19			18	20	20					56				
QC31	ChC	L			6	10	9										2					
QC37	YeP	L												300						100		
SK1	Wal	L																	3			
AB3	Bur	B												55								
AB6	MoW	B												16				11				
BC13	Dcr	H			10	12									3							

Table 18. Activity levels of the biochemical tracer, ethoxyresorufin *O*-deethylase (pmol/min/mg protein), in liver of 4 fish species reported by 3 mill studies. Values shown represent the mean, and standard deviation or range, sample size, and gender (male or female) fish sample.

	Reference			Study			Species
	Mean	±SD/range	N	Mean	±SD/range	N	
SK1	<7.5	<2-34	21	<16.2	<4-39	6	White sucker
	<5.6	<1-20	20	<6.7	<2-11	6	Walleye
AB5	1.5	0.7	10	1.3	0.8	3	White sucker(f)
	4.5	4.1	18	12.5	5.4	2	White sucker(m)
AB5	3.6	2.9	10	2.2	1.5	10	Longnose sucker(f)
	1.9	1.7	10	4.9	2.6	8	Longnose sucker(m)
AB7	No reference		49		ND-190	5	Mountain whitefish

Table 19. Number of chemicals analyzed for each organic chemical group, and the number and percent frequency of those detected at each site. Analyses for dioxins and furans are based on Cl_4-Cl_8 congeners.

Group	Data sets	Measured at each site	Detected at reference site	Detected at study site
Dioxins	34	238	27 (11%)	34 (14%)
Furans	33	340	30 (9%)	37 (11%)
Resin acids	63	600	95 (16%)	149 (25%)
Fatty acids	38	240	170 (71%)	162 (68%)
Cl phenols	14	384	13 (3%)	20 (5%)

Table 20. List of 44 of the 48 mills that monitored resin acids as tracers with information on their furnish, process type and primary or secondary treatment of their mill effluent.

Mill	Furnish[1]	Type[2]	Treatment[3]
NF2	100%SW	TMP	2nd - ASB
NF3	100%SW	TMP	1st - DAF/PC
NS2	100%SW	TMP	1st - SC
NB8	100%SW	GW	1st - PC
ON22	100%SW	GM	1st - PC
QC8	100%SW	K	1st - PC
QC30	100%SW	K	1st - PC
QC23	100%SW	K	1st - PC
QC27	100%SW	K	2nd - ASB
ON16	100%SW	K	2nd - ASB
AB6	100%SW	K	2nd - ASB
AB7	100%SW	K	2nd - ASB
BC9	100%SW	K	2nd - ASB
BC13	100%SW	K	2nd - CAS
QC36	100%SW	S	1st - PC
AB4	100%SW	CTMP	2nd - OAS
BC8	100%SW	CTMP	2nd - ASB/CAS
BC7	100%SW	K/CMP	2nd - ASB
NB6	100%SW	K/TIS	1st - SC
NS6	95%SW,5%HW	S	1st - PC
NB9	95%SW,5%HW	K	2nd - ASB
QC12	95%SW,5%HW	K	1st - PC
QC46	95%SW,5%PBP	GW	1st - PC
ON21	93%SW,7%PKP	GM&S	1st - PC
NB4	90%SW,10%HW	TMP	1st - PC
QC37	90%SW,10%HW	K	1st - SB
QC49	90%SW,10%HW	GW	1st - PC
NB3	85%SW,15%HW	GW&S	2nd - ASB
NS4	80%SW,20%HW	K	2nd - ASB
QC7	60%SW,40%HW	TMP	1st - SB
NB7	65%HW,35%SW	S	2nd - AN/AR
AB3	70%HW,30%SW	K	2nd - ASB
QC11	75%HW,25%SW	K/CO	1st - PC
AB5	80%HW,20%SW	CTMP	2nd - OAS
NB10	100%HW	K	2nd - ASB
NB11	100%HW	S	2nd - AN/AR
QC24	100%HW	K	2nd - ASB
QC31	100%SW/HW	S	2nd - ASB/OAS
QC13	60%WP,40%SW	TMP?	1st - DAF
ON9	40%WP,55%PKP,5%SW	TMP/GM	1st - PC
ON18	66%PKP,30%HW,2%WP	GM	2nd - ASB
QC14	100%WP	K/TMP	1st - PC
QC15	100%WP	GW	1st - PC
ON5	100%WP	LB	1st - PC
NS3	WP,PKP/100%WP	TMP/LB	1st - PC/SC
QC48	WP,SW,PBP	TMP/deink	1st - PC
QC35	PKP,SW	G/TMP	1st - PC
QC17	HW	P	1st - PC
QC34	SW,WP	TMP/deink	1st - PC
QC45	SW,WP	GW	?
NS1	SW/HW	TMP	1st - PC
QC50	SW/HW	CTMP	1st - SB
SK1	SW,HW	K	2nd - ASB

[1]Furnish: Waste paper (WP); Deink (D); Softwood (SW); Hardwood (HW); Purchased kraft pulp (PKP); Purchased bleach pulp (PBP); Rag (R)
[2]Mill Type: Groundwood (GW); Linerboard (LB); Thermochemical pulp mill (TMP); Chemithermomechanical pulp mill (CTMP); Tissue mill (TIS); Sulphite mill (S); Kraft mill (K); Corrugating mill (Co); Deinking facility (deink); Paper mill (P)
[3]Treatment: Primary. Primary clarifier (PC); Screening (SC); Dissolved air flotation (DAF); Sedimentation basin (SB).
Secondary. Aerated stabilization basin (ASB); Anaerobic/aerobic (AN/AR); Conventional activated sludge (CAS); Oxygen activated sludge (OAS).

Table 21. Concentrations of dehydroabietic acid in mill effluent, study and reference sites in relation to percent softwood, hardwood and other furnish reported by some mills. Furnish data not available for some mills. ND indicates concentrations were below the detection limit of 2 µg/L. NR indicates concentrations were not reported.

	Concentration, µg/L			Percent furnish		
Mill	Effluent	Study site	Reference site	SW	HWD	Other
QC12	930	ND	ND	95	5	
QC36	820	83	6	100		
QC35	690	9	6			
QC15	680	6	ND			100
QC13	660	ND	ND	40		60
QC45	170	ND	NR			
QC17	140	ND	ND			
QC7	99	NA	ND	60		40
QC14	83	NA	ND			100
QC30	24	ND	ND	100		
QC48	12	110	ND			
QC49	8	ND	ND	90	10	
QC19	6	NR	ND			
QC16	3	2	4			
QC46	3	2	2	95		
QC11	2	1	0.7	75	25	
QC23	NR	720	2	100		
QC34	ND	ND	ND			
QC38	ND	NR	NR			

Table 22. Concentrations of abietic acid in mill effluent, study and reference sites in relation to percent softwood, hardwood and other furnish reported by some mills. Furnish data was not available for some mills. ND indicates concentrations were below detection limit of 2 µg/L, and NR indicate data was not reported.

	Concentration, µg/L			Percent furnish		
Mill	Effluent	Study	Reference	SW	HWD	Other
QC36	5600	91	7	100		
QC12	3000	ND	ND	95	5	
QC35	2600	34	ND			
QC13	620	ND	ND	40		60
QC14	130	NR	ND	100		
QC11	2	1	1	75	25	
QC23	NA	300	ND	100		
QC46	ND	ND	ND	95		

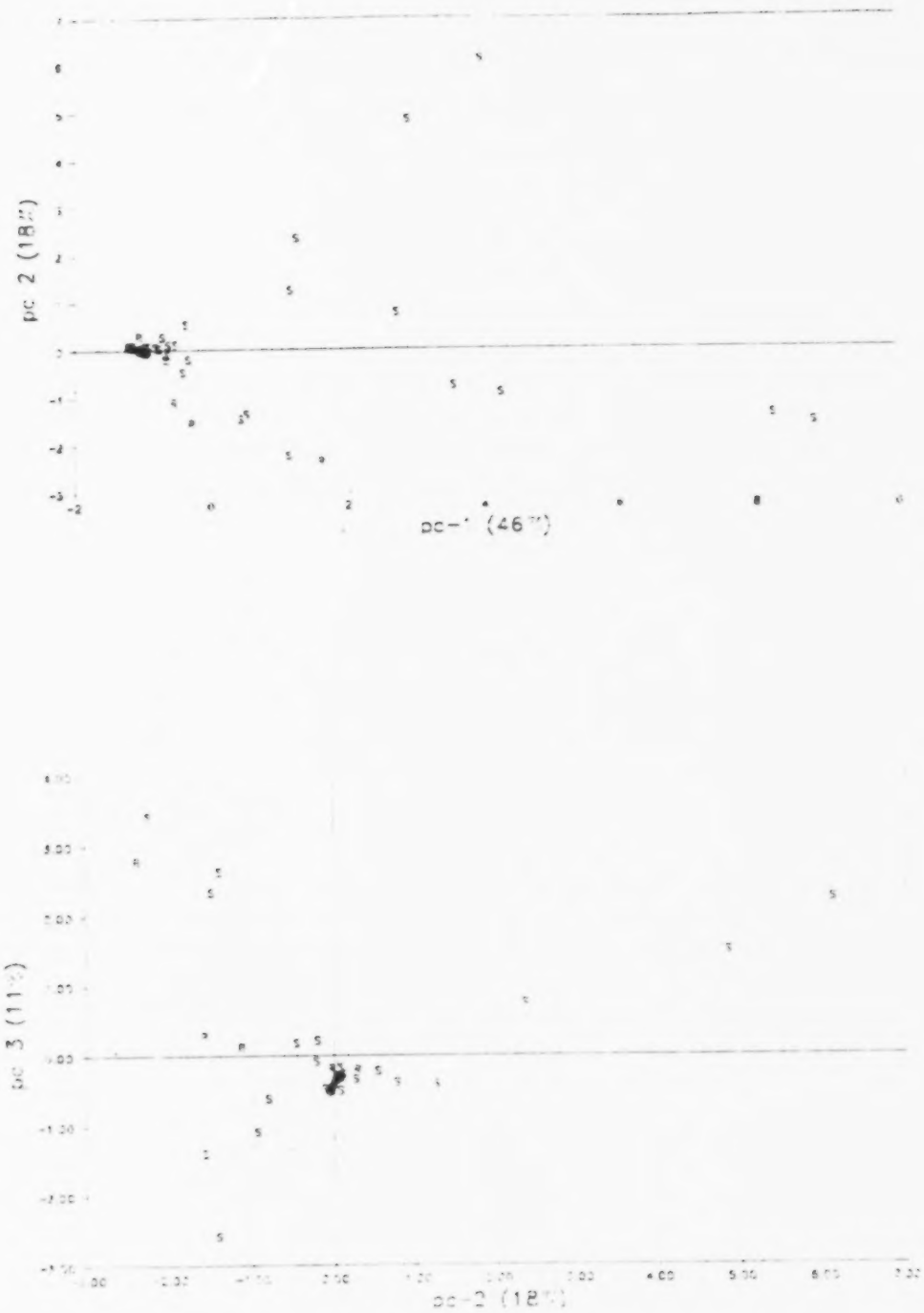


Fig. 1, 2. Resin acids in the bile of fish from reference ϟ and study (S) zones of the nine selected (see text) mills, projected on the plane of the principal components pc-1 and pc-2 which account for 46% and 18% of the original variance, respectively, and pc-2 and pc-3 (an additional 11% of the original variance). A reasonably good separation of the reference and study zones can be seen.

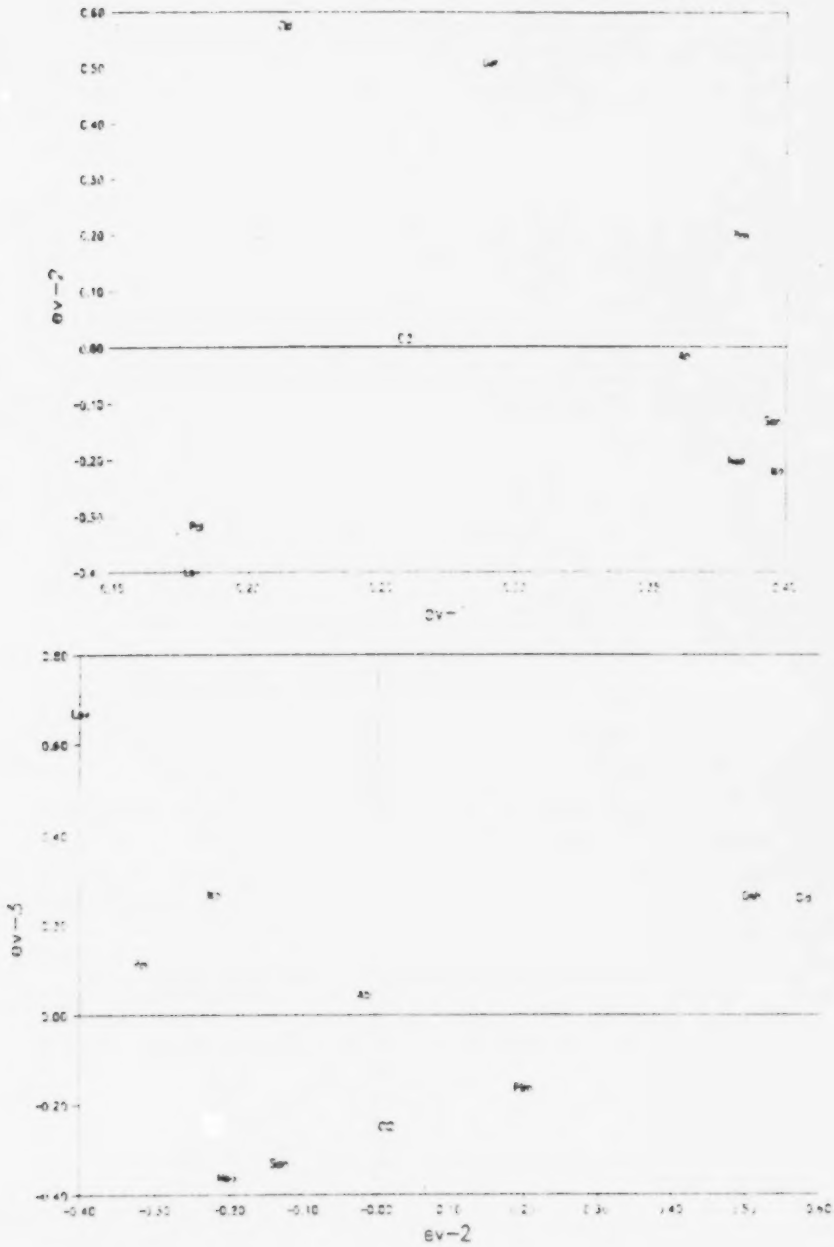


Fig. 3, 4. A 'loading' plot (see text) of resin acids in the bile of fish from reference and study zones of nine selected (see text) mills. It shows the effect of the individual resin acids on the principal components pc-1 and pc-2 (ev-1 and ev-2, respectively), and pc-2 and pc-3 (ev-2 and ev-3, respectively). The weights are designated by resin acid acronyms Abi=abietic, Cl2=dichlorodehydroabietic, Cld=chlorodehydroabietic, Deh=dehydroabietic, Iso= isopimaric, Lev=levopimaric, Neo=neoabietic, Pal=palustric, Pim=pimaric, San=sandaracopimaric acid). The distances between resin acids are inversely proportional to their correlations.

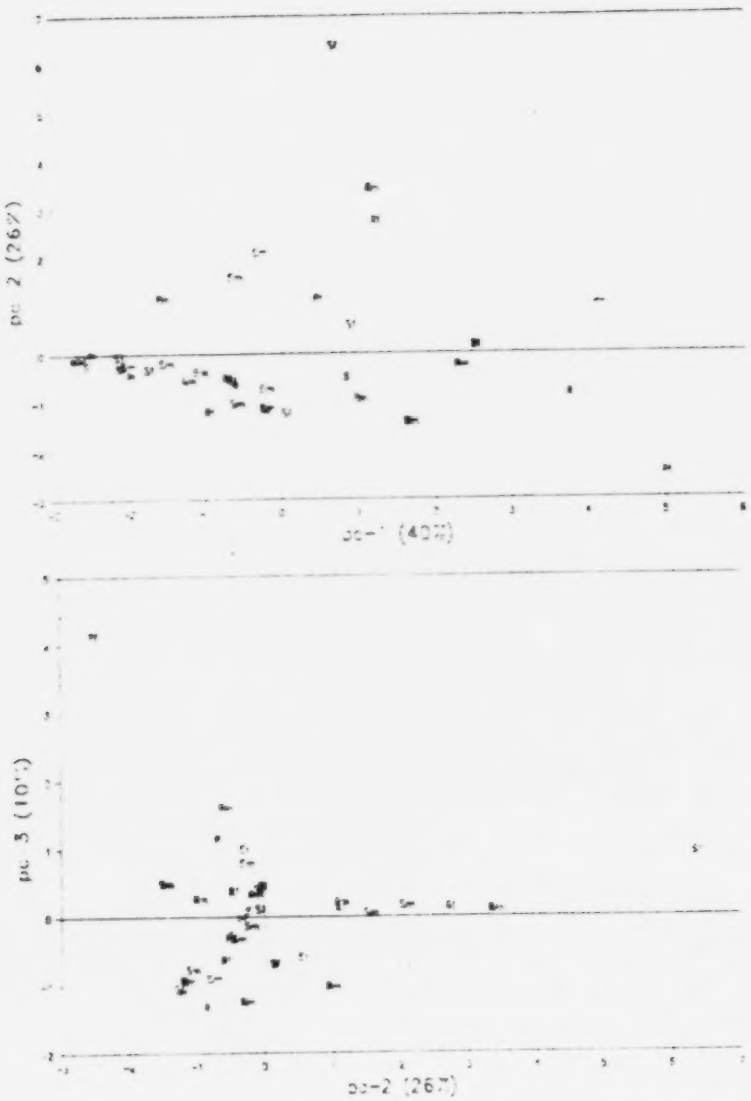


Fig. 5, 6. The measurement of nine fatty acids in the liver/gall bladder of tomcod and mummichog and in the hepatopancreas of green crab. The upper figure is projected on the plane of the first two principal components, pc-1 and pc-2, which account for 40% and 26% of the original variance, respectively (score plot, see text). Positions of samples are indicated by reference (R), and study (S) site, and male (m) and female (f). The distance between samples is inversely proportional to their similarity. The bottom figure is projected on the plane of principal components, pc-2 and pc-3, which account for 26% and 10% of the original variance, respectively.

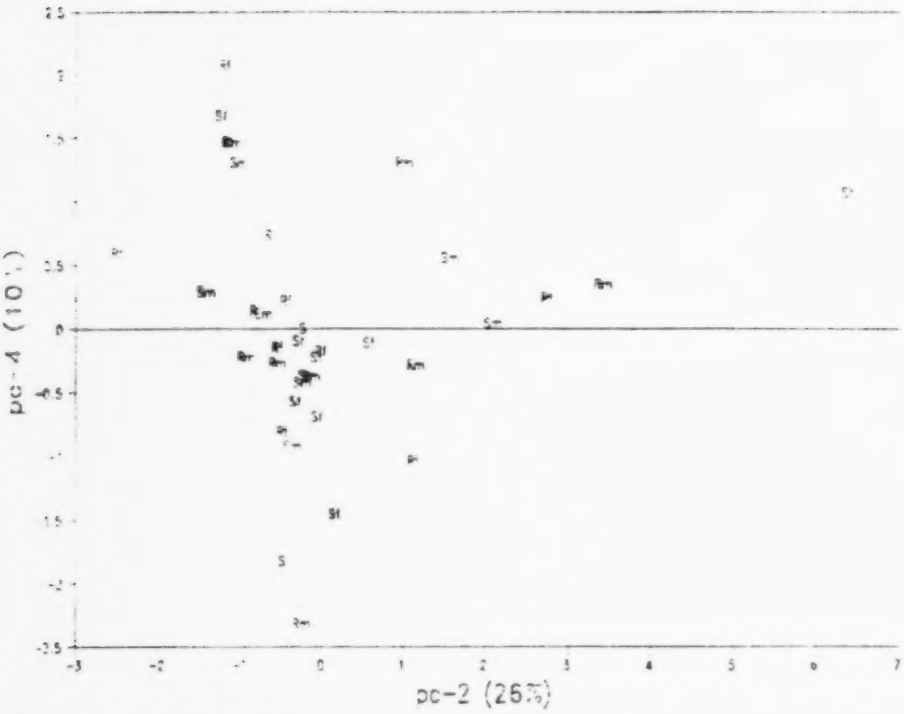


Fig. 7. The measurements of nine fatty acids in the liver/gall bladder of tomcod and mummichog and in the hepatopancreas of green crab. This figure is projected on the plane of principal components, pc-2 and pc-4, which account for 26% and 10% of the original variance. No separation between reference and study sites can be seen. Data sets were based on mills NB4, 5, 7 and 9.

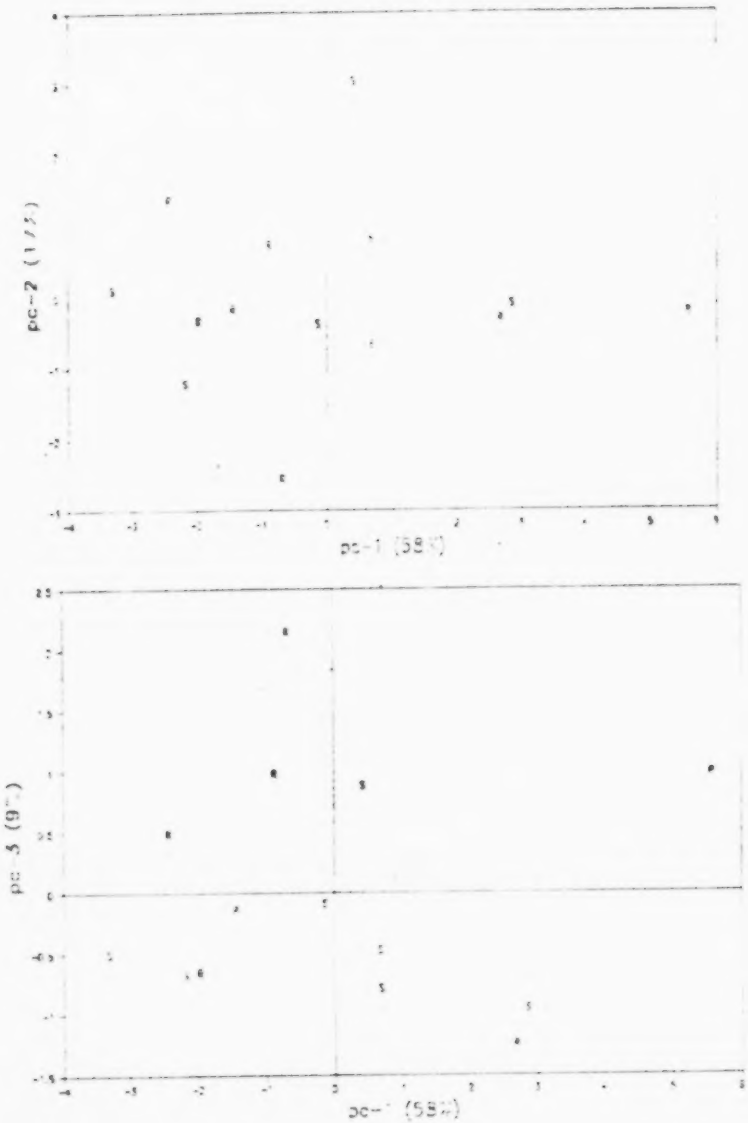
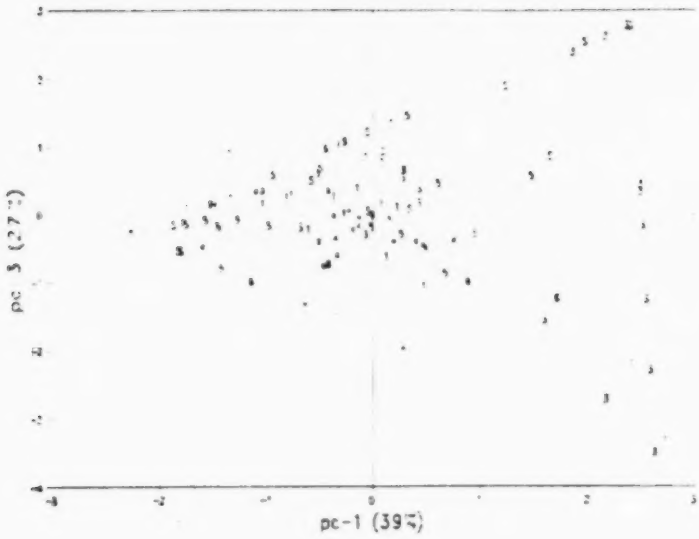


Fig. 8, 9. The measurements of nine fatty acids in the bile of white sucker, projected on the plane of the principal components, pc-1 and pc-2, which account for 58% and 17% of the original variance, respectively (score plot, see text), and pc-1 and pc-3 (additional 9% of variance). Positions of samples are indicated by R (reference zone), S (study zone). The distance between samples is inversely proportional to their similarity. No separation between reference and study zones can be seen.



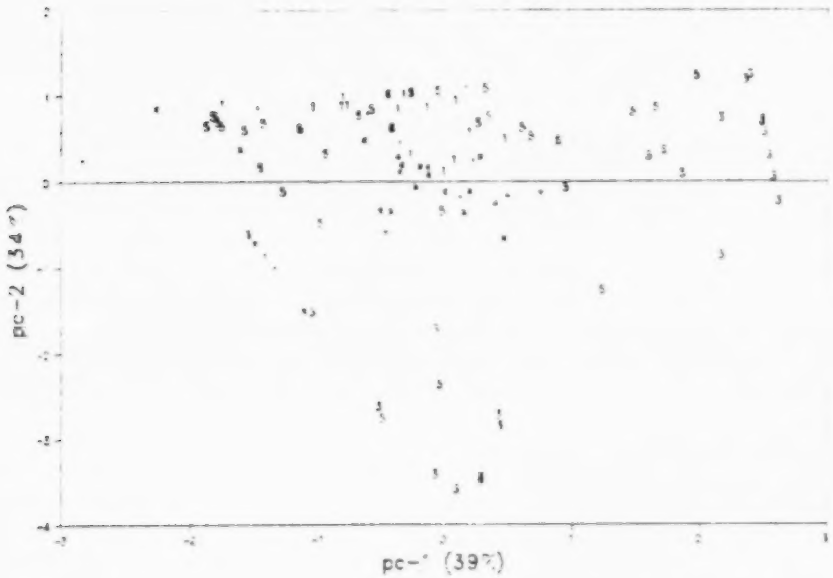


Fig. 10, 11. Resin acids (pimaric, isopimaric, abietic, and dehydroabietic acid) in the bile of fish, projected on the plane of the principal components pc-1 and pc-2, which account for 39% and 34% of the original variance, respectively, and pc-1 and pc-3 (an additional 27% of the original variance). Positions of samples are indicated by 'x' for literature data (see [3-7]), numbers 1,3,5, and 6 refer to the four laboratories performing the measurements in the Tracer Program. Nonrandom distribution of the laboratory results indicates systematic differences among the laboratories.

Appendix 1.

Working Group to Assess Tracer Issue
Terms of Reference
January 1996

Purpose: The regions and mills have expressed some difficulty with the requirement to select, and analyse for, tracers to substantiate exposure and non-exposure of fish to pulp and paper mill effluents in marine and freshwater receiving environments. This Working Group will assess the tracer issue and provide recommendations to *the EEM National Team, and to the EEM Technical Management Committee and to the EEM Steering Group.*

Membership:	Sue Humphrey/Nardia Ali	-	DOE, Downsview, ON
	Roy Parker	-	DOE, Dartmouth, NS
	Raymond Van Coillie	-	DOE, Montreal, QC
	Art Niimi	-	DFO, Burlington, ON
	Vladimir Zitko	-	DFO, St. Andrews, NB
	Craig Wood	-	Noranda Forest Inc., Pointe Claire, QC
	Isabelle Cloutier	-	Abitibi-Price Inc., Quebec City, QC

This core **Working Group** of 7-8 people will participate in monthly meetings/teleconferences. A number of additional individuals will be consulted for specific technical expertise when needed. For example, Art Niimi has already identified some scientists from different organizations, both government and non-government, who can provide technical input within specific areas.

Objectives:

1. Review the concept and purpose of using effluent tracers in the program.
2. Summarize the difficulties encountered by mills required to measure an effluent tracer in fish.
3. Document the situations where measuring effluent tracers in fish was not problematic.
4. Identify substances present in secondary treated effluent, as well as substances that can safely be added to effluent, that might be candidate tracers for future EEM studies.
5. Assess advantages/disadvantages for use of these tracers in mill water intake, treatment system, effluent outflow and receiving waters based on literature, ongoing research, available data.
6. Identify techniques for tracer measurement including information on availability of commercial analyses.
7. Recommend 3-5 tracers appropriate for different pulping /papermaking processes, marine/freshwater environments, shellfish. Document strengths/weaknesses of each.

Process: Identify roles of Working Group members, have at least monthly meetings/teleconferences to discuss progress and decide on further actions. Interested parties will be kept informed. Technical input from others, based on field experiences, will be welcome.

Time Period: December 1995 to October 1996

Report: A report summarizing the Working Group's results and recommendations will be prepared and distributed to the EEM National Team, to all pulp and paper mills, and to the *EEM Technical Management Committee and to the EEM Steering Group,* in October 1996.

Appendix 2. List of 134 Canadian mills that participated in the EEM studies. Each mill is identified by an alphanumeric code by province. The 76 mills that monitored tracers in biota are identified by an asterisk, and the 17 mills that submitted joint Reports are noted.

Newfoundland Mills

NF1	Abitibi-Price, Grand Falls
NF2*	Abitibi-Price, Stephenville
NF3*	Cornor Brook Pulp and Paper, Corner Brook

Nova Scotia Mills

NS1*	ABTCO, Canexel Div.
NS2*	Bowater Mersey Paper Com. Ltd., Liverpool
NS3*	CKF, Inc., Hantsport (Joint Report with NS5)
NS4*	Kimberly Clarke Maritimes, Pictou
NS5*	Minas Basin Pulp and Paper, Hantsport (Joint Report with NS3)
NS6*	Stora Forest Industries Ltd., Port Hawkesbury

New Brunswick Mills

NB1	Avenor Maritimes
NB2	Alcell Technologies Inc., Atholville
NB3*	Fraser Paper Inc., Edmundston
NB4*	Irving Paper Inc., Saint John
NB5*	Irving Pulp and Paper Ltd., Saint John (Joint Report with NB6)
NB6*	Irving Tissue Co., Saint John (Joint Report with NB5)
NB7*	Lake Utopia Pulp and Paper, St. George
NB8*	REPAP Groundwood Mill, Miramichi
NB9*	REPAP New Brunswick Inc., Kraft Mill, Miramichi
NB10*	St. Anne-Nackawic Pulp Co. Ltd., Nackawic
NB11*	Stone Container Inc., Bathurst Div., Bathhurst

Quebec Mills

QC1	Abitibi-Price Inc., Alma
QC2	Abitibi-Price Inc., Beaupre
QC3	Abitibi-Price Inc., Kenogami
QC4	Produits forestiers Alliance Inc., Dolbeau
QC5	Produits forestiers Alliance Inc., Donnaconna
QC6	Avenor Inc., Gatineau
QC7*	BPCO, d'Emco Div. Ltd., Pont Rouge
QC8*	Cartons Saint-Laurent Inc., La Tuque
QC9	Cartons Saint-Laurent Inc., Matane
QC10	Cascades, Breakeyville
QC11*	Cascades Cabano Inc.
QC12*	Cascades East Angus Inc., East Angus (Joint Report with QC52)
QC13*	Cascades Inc., Joliette Div., Joliette
QC14*	Cascades, Jonquiere
QC15*	Cascades Inc., Kingsey Falls
QC16*	Cascades Lupel Inc., Cap de la Madeleine (Joint Report with QC21)
QC17*	Rolland Inc., Papers Div., St. Jerome
QC18	CDM Lamine, Drummondville
QC19	Compagnie Gaspesia Ltd., Abitibi-Price Inc., Chandler
QC20	Daishowa, Quebec
QC21*	Desencrage C.D.M., Cap de la Madeleine (Joint Report with QC16)
QC22	Domtar, Beauharnois
QC23*	Domtar, Kraft Mill Div., Lebel sur Quevillon
QC24*	Domtar, Windsor
QC25	Donohue, Inc., Amos
QC26	Donohue, Inc., Clermont
QC27*	Donohue, Inc., St. Felicien
QC28	Donohue, Inc., Matane
QC29*	E.B. Eddy, Ltd., Hull (Joint Report with QC53 and ON11)
QC30*	Emballages Stone Inc., Chaleurs Div., New Richmond
QC31*	Emballages Stone Inc., Pontiac Div., Portage du Fort
QC32	F.F. Soucy, Riviere du Loup
QC33	J. Ford Ltd., Portneuf

QC34* Kruger Inc., Bromptonville
QC35* Kruger Inc., Trois Riveres
QC36* J. Maclaren Inc., Masson
QC37* J. Maclaren Inc., Kraft Mill Div., Thurso
QC38* Papiers Perkins Inc., Lachute
QC39 Port Cartier, Port Cartier
QC40 Produits Desbiens Inc., Desbiens
QC41 Malette Quebec Inc., St. Raymond
QC42 QUNO, Baie Comeau Operations, Baie Comeau
QC43 Papiers Scott Ltd., Crabtree
QC44 Papiers Scott Ltd., Lennoxville
QC45* St. Raymond, Mohawk Pulp Mill, St. Antonin
QC46* Stone Consolidated Corp., Laurentide Div., Grand Mere
QC47 Stone Consolidated Corp., Port Alfred Div., La Baie
QC48* Stone Consolidated Corp., Belgo Div., Shawinigan
QC49* Stone Consolidated Corp., Wayagamack Div., Trois Rivieres
QC50* Tembec Inc., Temiscamingue
QC51 Tripap Inc., Trois Rivieres
QC52* Cascades Cartech Inc. (Joint Report with QC12)
QC53* Scott Paper, Hull (Joint Report with QC29 and ON11)

Ontario Mills

ON1 Abitibi-Price Inc., Iroquois Falls
ON2 Abitibi-Price Inc., Thunder Bay
ON3* Avenor Inc., Dryden
ON4* Avenor Inc., Thunder Bay
ON5* BeaverWood Fibre Co., Thorold
ON6* Domtar Packing Inc., Red Rock
ON7 Domtar Packing Inc., Trenton
ON8* Domtar Specialty Fine Papers Inc., Cornwall
ON9* Domtar Specialty Fine Papers Inc., St. Catharines (Joint Report with ON14, ON19 and ON27)
ON10* E.B. Eddy Forest Products Ltd., Espanola
ON11* E.B. Eddy Forest Products Ltd., Ottawa (Joint Report with QC29 and QC53)
ON12* James River-Marathon Ltd., Marathon
ON13 Kimberley-Clark Inc., Huntsville
ON14* Kimberley-Clark, Inc., St. Catharines (Joint Report with ON9, ON19 and ON27)
ON15* Kimberley-Clark Forest Products Inc., Terrace Bay
ON16* Malette Kraft, Smooth Rock Falls
ON17 MacMillian Bloedel Ltd., Sturgeon Falls
ON18* Provincial Papers, Thunder Bay
ON19* QUNO Corp., Thorold (Joint Report with ON9, ON14 andON27)
ON20* Stone Consolidated Corp., Fort Frances
ON21* Stone Consolidated Corp., Kenora
ON22* St. Mary's Paper Ltd., Sault Ste Marie
ON23* Spruce Falls Inc., Kapuskasing
ON24 Sonoco Ltd., Trenton
ON25 Strathcona Paper Co., Napanee
ON26 Thunder Bay Packaging Co., Thunder Bay
ON27* Thorold Specialty Papers Inc., Thorold (Joint Report with ON9, ON14 and ON19)

Manitoba Mills

MB1 Repap Manitoba Inc., The Pas
MB2 Pine Falls Paper Co., Pine Falls

Saskatchewan Mills

SK1* Weyerhauser Saskatchewan Ltd., Prince Albert

Alberta Mills

AB1 Alberta Newsprint, Whitecourt
AB2 Alberta Pacific Forest Industries, Inc., Boyle
AB3* Daishowa-Marubeni International Ltd., Peace River
AB4* Millar Western Pulp Ltd., Whitecourt
AB5* Slave Lake Pulp Corp., Slave Lake
AB6* Weldwood of Canada Ltd., Hinton
AB7* Weyerhauser Canada Ltd., Grande Prairie

British Columbia Mills

BC1*	Avenor, Inc., Gold River Operations, Gold River
BC2	Canadian Forest Products, Canfor, Prince George
BC3	Cariboo Pulp and Paper, Quesnel
BC4	Celgar, Castlegar
BC5	Crestbrook Forest Products, Skookumchuck
BC6*	Crown Packaging Ltd., Paperboard Div., Burnaby
BC7*	Eurocan Pulp and Paper, Kitimat
BC8*	Fibreco, Taylor
BC9*	Finlay Forest Products, Finlay (Joint Report with BC12)
BC10	Fletcher Challenge Canada Ltd., Crofton Pulp and Paper Div., Crofton
BC11	Fletcher Challenge Canada Ltd., Elk Falls Pulp Div., Campbell River
BC12*	Fletcher Challenge Canada Ltd., Mackenzie (Joint Report with BC9)
BC13*	Harmac Pacific, Harmac Pulp Mill, Nanaimo
BC14	Howe Sound Pulp and Paper Ltd., Port Mellon
BC15	Macmillan Bloedel Ltd., Alberni Pulp and Paper Div., Port Alberni
BC16	Macmillan Bloedel Ltd., Powell River Pulp Div., Powell River
BC17	Newstech Recycling, Coquitlam
BC18	Northwood Pulp and Paper, Prince George
BC19	Quesnel River Pulp Co., Quesnel
BC20	Scott Paper, New Westminster
BC21	Skeena Cellulose Inc., Skeena Pulp Operations, Prince Rupert
BC22	Western Pulp Ltd. Partnership, Port Alice Pulp, Port Alice
BC23	Western Pulp Ltd. Partnership, Woodfibre Pulp Operations, Squamish
BC24	Weyerhauser Canada, Kamloops

Appendix 3. Analytical laboratories used for dioxin (DD), furan (DF), resin acids (RA), fatty acids (FA) and chlorinated phenolics (CP) analyses in EEM studies. Mill codes area identified in Appendix 1, and analytical laboratories that conducted the analyses included Canviro (C); Enviro-Test (E); Fenwick (F); Novamann (N); Wellington (W); and Zenon (Z).

Mill	DD	DF	RA	FA	CP
NF2			N	N	
NF3			N	N	
NS1			N	N	
NS2			N	N	
NS4			N	N	
NS5			N	N	
NS6	W	W	E	E	
NB3	C	C	E		
NB4			F	F	
NB5	F	F	F	F	
NB7			F	F	
NB8			F	F	
NB9	F	F	F	F	
NB10	Z	Z	N	N	
NB11			N	N	
QC7			E		
QC8	Z	Z			
QC11			Z	Z	
QC12			Z	Z	
QC13			Z	Z	
QC14			E		
QC15			Z	Z	
QC16			Z		
QC17			Z	Z	
QC23	Z	Z	Z		
QC24	Z	Z			
QC27	W	W			
QC30			N		
QC31	Z	Z		N	
QC34			Z	Z	
QC35			Z	Z	
QC36			E		
QC37	W	W	E		E
QC45			Z	Z	
QC46			N		
QC49	Z	Z	N		N
QC50	W	W	E		
ON3	Z	Z			
ON4	Z	Z			
ON5			N	N	
ON6	W	W			
ON8	W	W			
ON9	W	W	E		
ON10	Z	Z			
ON12	Z	Z			
ON15	Z	Z			
ON16			N	N	N
ON18			N	N	
ON20	E	E			
ON21			N	N	
ON22			N	N	
ON23	W	W			
SK1	E	E	E		E
AB3	E	E	E		E
AB4			E		
AB5			E		
AB6	E	E	E		E
AB7	E	E	E		
BC1	Z	Z			
BC6			Z		
BC7			Z	Z	
BC8			E		
BC9	E	E	E		E
BC13					Z

Appendix 4.

Aquatic Toxicity Workshop: Special Session on Tracers

A special session on tracers was held at the 23rd Annual Aquatic Toxicity Workshop in Calgary, October 6-9, 1996. The session was divided into three activities that included presentations by invited international speakers and the Tracer Working Group, two poster presentations and a panel discussion session.

Dr. Jaakko Paasivirta, Department of Chemistry, University of Jyvæskylæ, Finland, presented "Organic trace compounds in fish and incubated mussels from pulp mill recipient waters." The study determined suitable organic tracers using incubated mussels and fish tissues to evaluate bioaccumulation capacity and compare it to the different compounds found in the effluent and the receiving waters. Discharges of most bleaching pulp mills contained organochlorine compounds which accumulated in the receiving waters. Biomethylation products of chlorophenols, chloroanisoles and chloroveratroles cause a bad taste in fish exposed to bleaching pulp. Chlorophenols and their derivatives were not related only to pulp mill effluent discharges. Chloroveratroles were, however, most characteristic to pulp mill origin as well as aromatic chlorocarbons especially chlorocymenes. Chlorinated alkyl bibenzyls (stilbene derivatives), alkyl phenanthrenes (especially retenes) and alkyl naphthalenes were also characteristic of a bleaching pulp mill.

Dr. Trevor Stuthridge, New Zealand Forest Research Institute, Rotorua, New Zealand, presented the paper "Application of resin acids as indicators of environmental exposure to pulp and paper mill waste discharges." This study reported bioaccumulation factors for resin acids in bile was several orders of magnitude greater than those observed in other tissues. A good linear relationship was shown between tissue concentration factors in water and fish tissues.

Dr. Vladimir Zitko, Department of Fisheries and Oceans, St. Andrews, NB, and Roy Parker, Environment Canada, Dartmouth, NS, presented an update and interim findings of the Tracer Working Group.

Poster presentations included "Verifying the exposure of fish to effluent by chemical tracers at bleached-kraft pulp mills employing secondary treatment technology" by Chris Fraikin, Golder Associates, Calgary, AB, that presented the results of improvements to effluent quality and the subsequent decline in the concentrations of tracer compounds at the pulp and paper mill at Grande Prairie, Alberta. Bleaching and secondary treatment resulted in low concentrations of compound bioaccumulation in fish tissues. Environmental transport mechanisms should be considered since many tracer compounds are non detectable in water. Among-species variation demonstrates that species-specific factors greatly influence the ability of fish to bioaccumulate mill-related compounds. The induction of hepatic EROD activity can be a useful measure of exposure to mill effluents, but its usefulness as a tracer is species-specific.

Patrick Shaw, Environment Canada, Vancouver, BC, presented the poster "Organochlorine contaminants in fish from the Fraser River Basin." Peamouth chub (Mylocheilus caurinus) and mountain whitefish (Prosopium williamsoni) were sampled and measured for levels of organic and metal contaminants in tissues on the Fraser, Nechako and Thompson Rivers. The preliminary analysis showed low levels of contamination, with patterns of contamination related to upstream effluent sources.

A panel discussion session was held after the presentations. The panel was chaired by Roy Parker and included Craig Wood and Isabelle Cloutier of the Tracer Working Group, Dr. Paasivirta, Dr. Stuthridge, C. Fraikin and Dr. Deib Birkholz, Enviro-Test Laboratories, Edmonton, AB. The key issues that were discussed in this session are presented below:

Part of this session was organized as an open forum where panel members responded to questions from the floor. The key question and discussions are summarized in this section.

1. How many mills used tracers?

Half of the 134 mills studied tracers. The only cases where mills did not use tracers were if there was a physical barrier between the exposure and the reference zones such as a dam.

2. Why the need to study tracers?

The purpose of the tracer study (as stated in the EEM Program) is to determine and document if fish have been exposed to the effluent, and to prove they were collected in the exposure zone. Unless we cage the fish in the effluent stream, we have no idea whether or not the fish had been living there.

It is in the industry's best interest to have a type of marker (tracer) in order to show that the impaired fish are not necessarily due to exposure.

3. If, by using a tracer, we realize that 40 % of fish are not exposed, what would it mean to the study design?

Ideally, it would mean that the 40% not exposed should not be considered in the study.

4. Is there a variability due to different laboratory methods?

Six laboratories did the tracer analysis for the industry. No standard method was given to laboratories for some chemical analyses. There is a need for a standard reference in future studies.

5. Is there a variability due to field work?

Many mills did not sample the effluent and the fish at the same time. In some cases, fish were collected at two different times in the year as opposed to once a year for two years.

6. Should we look at other tracers from other sources than pulp and paper effluent?

Pulp and paper effluents are often located in urban areas. In order to separate the effects of the pulp and paper effluent from other sources, we need to identify the presence of these tracers.

7. What about adding a benign tracer to the effluent?

May not be possible due to other regulations such as Health and Welfare Canada.

8. What could be a good tracer?

A good indicator of long term exposure in fish population could be assimilation of carbon from the mill in biochemical molecules, so that a different isotope profile is found downstream from the mill. However, there are problems related to stable isotope studies in cases such as dam recycling effects which cause stable isotope formation.

Resin acids are present after primary treatment but in low concentrations. Levels after secondary treatment in the effluent are 0.0002 mg/L and they could be much less in the receiving waters. We need longer lived tracers than fatty and resin acids because of the variability of fishing periods. The solution could be to take measurements over time.

We need a specific tracer. Chloroline and its degradation products such as benaline, which is a good bioaccumulator, could be studied.

One could calculate the ratio between the concentrations in fish and concentrations in their muscles. Muscle are good in that they exhibit site specific effects. Compounds such as chlorophenols have half lives that are

greater in particles than in water, and greater still in sediments. This is why substances such as bioacids accumulate in sediments.

There are promising candidates on the extractive site, such as cytosterols, and camsterols, that could be applied, yet analysis in-field has not been conducted.
9. Should tracers be there to interpret problems?

One would not be able to determine the causes and effects. Tracers indicate exposure. Dose and response must be determined in laboratories. The environment is too complex to separate causes and effects.

Problem is that one cannot be sure if compound concentrations are due to long-term exposure to small concentrations or short-term exposure to large concentrations. Research needs to be done into how each fish species metabolizes the compounds and the nature of the concentrations of short term exposure to large concentrations.

Tracers, while not the final proof of cause which may be due to environmental variables, can still be very helpful in interpretations.

Focus may be in the wrong area. Effluent samples look at the water/liquid phase of the effluent yet the compounds are being bioaccumulated in the fish and not necessarily coming out of the dissolved phase of the effluent. We should look at suspended sediments.

10. Tracer Working Group should change its focus.

The Group needs to focus on why we need tracers in the first place. Tagging fish would result in better knowledge of where the fish are going. We need to refocus on the biology.

The only real problem seems to be endocrine disruption. In cycle 2, we should work with B-cytosterols and nonylphenols. However, these are not part of the EEM Program, which is monitoring. Research takes place outside.

The industry is going to focus on continued environmental improvement. The industry is spending a lot of money and wants to document its improvement.
Comments received after the panel discussion session:

The Group should consider the possibility of using surrogate species that are easier to monitor and could provide more useful information.

Even if appropriate tracers could be identified, the issue of exposure time and position will remain problematic with mobile species. The real issue is not the utility of tracers but the utility of monitoring mobile fish.

The Group should give serious consideration to using caged bivalves for monitoring exposure and effects associated with pulp and paper mill effluents.

Appendix 5. Fish Tracer Decision Tree

Goal: To determine if the measurement of effluent tracers in fish should be a component of the Pulp and Paper EEM Program.

1: Will the mill be required to conduct a fish survey in Cycle 2?

No: Fish tracer*(s)* not applicable
Yes: Go to 2

2: Can the fish move freely between the exposure and reference zones? *(For example, there is a dam or similar barrier between the exposure and reference zone)*

No: Fish tracer(s) not applicable
Yes: Go to 3

3: Is the mill's furnish at least 50% softwood, aspen or recycled fibre?

No: Fish tracer*(s)* not applicable
Yes: Go to 4

4: Are resin acids[1] present in sufficient *concentration* in the mill effluent? (see example calculation attached).

No: No other suitable tracer is available at this time; therefore the measurement of a fish tracer is not required.
Yes: Go to 5

5. Measure resin acids in samples of effluent, receiving water and fish bile.

Note 1: Other tracers maybe substituted if proven to be effective.

Sample Calculation

To determine if the mill effluent contains sufficient concentrations of resin acid to use as a tracer.

Assumptions:
1) Detection limit for resin acids in fish bile = 0.5 μg/g
2) Detection limit for resin acid in water samples = 25 μg/L
3) Bioconcentration factor (BCF) of resin acids in fish bile = 1000
4) Exposure zone fish are captured within the 1% effluent plume

Equations: Tissue concentration = water concentration X BCF

Water concentration = effluent concentration X 0.01

Therefore: Effluent concentration = tissue concentration / (0.01 X BCF)

Effluent Concentration = 0.5 μg/g / (0.01 X 1000)
= 0.05 μg/g
= 50 μg/L

Therefore if the effluent contains 50 μg/L of resin acid, then there should be detectable concentrations of resin acid in the fish bile. This is a very conservative estimate. Detection limits for resin acids in bile can be as low as 0.1 μg/g and BCF for resin acids in fish bile have been reported in the 10^4 to the 10^6 range (Stuthridge, et al. 1995).